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PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA BÁSICA E APLICADA

ACURÁCIA DIAGNÓSTICA DOS NOVOS BIOMARCADORES NA NEFRITE LÚPICA: UMA *OVERVIEW* DE REVISÕES SISTEMÁTICAS

JULIANA DE ANDRADE REBOUÇAS GUIMARÃES

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Imunologia Básica e Aplicada do Instituto de Ciências Biológicas da Universidade Federal do Amazonas, como requisito para obtenção do título de Doutora em Imunologia Básica e Aplicada.

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RESUMO

Introdução: O Lúpus eritematoso sistêmico (LES) é uma doença autoimune crônica, com envolvimento inflamatório multiorgânico e mortalidade que chega a ser 2,6 vezes mais alta que a população geral para a mesma faixa etária e sexo. Aproximadamente 50% dos pacientes com LES desenvolvem comprometimento renal (nefrite lúpica). A demora no diagnóstico da nefrite lúpica está associado a um maior risco de progressão para doença renal terminal, com necessidade de terapia de substituição e maior mortalidade. As manifestações clínicas iniciais da nefrite lúpica são frequentemente discretas ou ausentes, sendo usualmente detectadas por meio de exames complementares. Apesar de serem amplamente utilizados na prática clínica, sua acurácia é limitada. Um grande esforço científico tem sido feito em busca de biomarcadores mais sensíveis e específicos nos últimos anos. Algumas revisões sistemáticas avaliaram individualmente alguns dos novos biomarcadores estudados em pacientes com nefrite lúpica (NL). Esta *Overview* teve o objetivo de sumarizar as revisões sistemáticas (RS) sobre a acurácia de novos biomarcadores séricos e urinários para diagnosticar nefrite lúpica em pacientes com LES, discutindo como nossos resultados podem guiar o manejo clínico da doença e o direcionamento da pesquisa nesta área. **Métodos:** A pergunta da pesquisa é "Qual a acurácia diagnóstica dos novos biomarcadores séricos e urinários estudados para o diagnóstico da nefrite lúpica nos pacientes com LES?". Nossa busca incluiu revisões sistemáticas de estudos observacionais avaliando a acurácia diagnóstica de novos biomarcadores séricos ou urinários para nefrite lúpica. As seguintes bases de dados foram pesquisadas: PubMed, EMBASE, BIREME/LILACS, Scopus, Web of Science, and Cochrane, incluindo a literatura cinzenta via Google Scholar e PROQUEST. Dois autores analisaram as revisões para inclusão, extração de dados e avaliação do risco de viés (ferramenta ROBIS). **Resultados:** Onze RSs sobre acurácia diagnóstica de novos BMs séricos e urinários na NL foram selecionados. As RSs avaliaram 7 BMs distintos: (a) anticorpos (anti-Sm, anti-RNP, and anti-C1q), (b) citocinas (TWEAK and MCP-1), (c) uma quimiocina (IP-10), e (d) uma glicoproteína de fase aguda (NGAL), em um total de 21 braços de revisão (10 analisaram BMs séricos, e 13 analisaram BMs na urina). A população avaliada nos estudos primários foi predominantemente adulta. Duas RSs incluíram estritamente adultos, 6 revisões também incluíram estudos na população pediátrica, e 4 não reportou a faixa etária avaliada nos estudos primários incluídos. Os resultados da avaliação com a ferramenta ROBIS mostrou que a maioria das revisões tiveram um baixo risco geral de viés. **Conclusões:** Existem 11 RSs sobre a acurácia diagnóstica de novos biomarcadores séricos e urinários na nefrite lúpica. Entre os BMs avaliados, anti-C1q, urinary MCP-1, TWEAK, e NGAL se destacaram, enfatizando a necessidade de pesquisa adicional, especialmente focando em painéis diagnósticos para NL, e procurando corrigir questões metodológicas dentro do campo da pesquisa de acurácia diagnóstica. Isto permitiria uma melhor compreensão de sua utilidade e possivelmente auxiliaria na validação de seu uso clínico no futuro. **Registro:** Este projeto está registrado na base de dados *International Prospective Registry of Systematic Reviews* (PROSPERO) (CRD42020196693).

Palavras-chave

Nefrite lúpica; Biomarcadores; Overview

ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with multiorgan inflammatory involvement and a mortality rate that is 2.6-fold higher than individuals of the same age and sex in the general population. Approximately 50% of patients with SLE develop renal impairment (lupus nephritis). Delayed diagnosis of lupus nephritis is associated with a higher risk of progression to end-stage renal disease, the need for replacement therapy, and mortality. The initial clinical manifestations of lupus nephritis are often discrete or absent and are usually detected through complementary tests. Although widely used in clinical practice, their accuracy is limited. A great scientific effort has been exerted towards searching for new, more sensitive, and specific biomarkers in recent years. Some systematic reviews have individually evaluated new serum and urinary biomarkers tested in patients with lupus nephritis. This overview aimed to summarize systematic reviews on the accuracy of novel serum and urinary biomarkers for diagnosing lupus nephritis in patients with SLE, discussing how our results can guide the clinical management of the disease and the direction of research in this area. **Methods:** The research question is "*What is the accuracy of the new serum and urinary biomarkers studied for the diagnosis of LN in patients with SLE?".* We searched for systematic reviews of observational studies evaluating the diagnostic accuracy of new serum or urinary biomarkers of lupus nephritis. The following databases were included: PubMed, EMBASE, BIREME/LILACS, Scopus, Web of Science, and Cochrane, including gray literature found via Google Scholar and PROQUEST. Two authors assessed the reviews for inclusion, data extraction, and assessment of the risk of bias (ROBIS tool). **Results:** Eleven SRs on the diagnostic accuracy of new serum and urinary BMs in LN were selected. The SRs evaluated 7 distinct BMs: (a) antibodies (anti-Sm, anti-RNP, and anti-C1q), (b) cytokines (TWEAK and MCP-1), (c) a chemokine (IP-10), and (d) an acute phase glycoprotein (NGAL), in a total of 21 review arms (10 that analyzed serum BMs, and 13 that analyzed BMs in urine). The population evaluated in the primary studies was predominantly adults. Two SRs included strictly adults, 6 reviews also included studies in the paediatric population, and 4 did not report the age groups. The results of the evaluation with the ROBIS tool showed that most of the reviews had a low overall risk of bias. **Conclusions:** There are 11 SRs of evidence relating to the diagnostic accuracy of serum and urinary biomarkers for lupus nephritis. Among the BMs evaluated, anti-C1q, urinary MCP-1, TWEAK, and NGAL stood out, highlighting the need for additional research, especially on LN diagnostic panels, and attempting to address methodological issues within diagnostic accuracy research. This would allow for a better understanding of their usefulness and possibly validate their clinical use in the future. **Registration:** This project is registered on the International Prospective Registry of Systematic Reviews (PROSPERO) database (CRD42020196693).

Keywords

Lupus nephritis; Biomarkers; Overview

SUMÁRIO

1 INTRODUÇÃO

O Lúpus eritematoso sistêmico (LES) é uma doença inflamatória, autoimune crônica, multiorgânica, caracterizada por períodos de exacerbação e remissão. Sua distribuição é universal, com incidência e prevalência variando de um a 10 casos e 20 a 70 casos por 100.000 indivíduos ao ano respectivamente (Pons-Estel, Catoggio et al. 2015). O LES pode comprometer qualquer faixa etária e afetar tanto homens como mulheres. Contudo, acomete mais o sexo feminino, chegando a ser até 13 vezes mais comum em mulheres, com uma incidência maior durante a menacme, especialmente entre os 15 e 35 anos (Danchenko, Satia et al. 2006).

A etiologia do LES é desconhecida, e sua patogênese não pode ser explicada de forma completa por uma única causa. O conceito de que esta doença decorre de uma complexa interação entre fatores genéticos, epigenéticos e ambientais (obesidade, hormônios sexuais, luz ultravioleta (UV), tabagismo, infecções intercorrentes, etc) é o que constitui o paradigma fisiopatogênico atual (Mahajan, Herrmann et al. 2016).

Apesar da lacuna de conhecimento ainda existente sobre as vias celulares e moleculares envolvidas no distúrbio imune, que culmina clinicamente com o LES; a informação sobre os mecanismos envolvidos em sua patogênese, têm crescido nos últimos anos; e envolve ambas as imunidades inata e adaptativa (Fan, Liu et al. 2014, Sisirak, Ganguly et al. 2014, Moulton and Tsokos 2015, Goropevšek, Gorenjak et al. 2017, Lee, Bannan et al. 2017, Piantoni, Regola et al. 2018, Zhu, Yin et al. 2018). A característica marcante desta resposta alterada é a produção de autoanticorpos e um importante aumento na resposta IFN tipo

1-dependente e do sistema *B cell activanting factor* (BAFF) / *B lymphocyte stimulator* (BLyS)(Blanco, Palucka et al. 2001, Petri, Singh et al. 2009, Salazar-Camarena, Ortiz-Lazareno et al. 2016, Steri, Orrù et al. 2017).

O quadro clínico do LES é bem variável, podendo comprometer virtualmente qualquer tecido corporal (pele, trato gastrointestinal, articulações, pulmões, sistema nervoso, etc). Desta forma, os indivíduos que apresentam a doença, podem evoluir de formas bem distintas, e apresentar desde quadros clínicos brandos até extremamente graves, com risco de óbito (Ballou, Khan et al. 1982, Levy and Kamphuis 2012, Sternhagen, Bettendorf et al. 2022).

O comprometimento renal pela doença é denominado nefrite lúpica (NL) e chega a acometer 60% dos indivíduos com LES em determinado momento da doença. O impacto deste comprometimento no curso clínico da doença é importante, conferindo uma menor qualidade de vida e sobrevida neste subgrupo de pacientes quando comparados aos indivíduos sem nefrite. Apesar dos desfechos clínicos terem melhorado para a NL nas últimas décadas, cerca de 43% dos pacientes com classe IV e 20% com classe V ainda evoluem para doença renal terminal (DRT), sendo estes desfechos piores em pacientes de certos grupos étnicos, como afroamericanos e hispânicos (Gasparotto, Gatto et al. 2020).

A remissão clínica completa se correlaciona fortemente com a sobrevida renal a longo prazo, o que faz do diagnóstico precoce um objetivo a ser alcançado. Atualmente, o padrão-ouro para o diagnóstico de NL é a biópsia renal com análise histopatológica do material, e o monitoramento da NL, se faz com base em parâmetros clínicos e laboratoriais (pressão arterial, peso corporal,

creatinina sérica, complemento sérico, anti-DNAds, urinálise, proteinúria, etc) (Fanouriakis, Kostopoulou et al. 2019). Apesar de serem amplamente utilizados e auxiliarem no manejo clínico destes pacientes, se por um lado a biópsia é um procedimento invasivo que carreia seus próprios riscos, por outro, os exames laboratoriais utilizados no monitoramento, não possuem a performance diagnóstica ideal. Fazem-se necessários novos biomarcadores que suplantem a acurácia diagnóstica e efetividade dos biomarcadores atualmente existentes e que possibilitem um diagnóstico mais precoce, um melhor manejo clínico e auxiliem satisfatoriamente na pesquisa clínica desta área.

2 REFERENCIAL TEÓRICO

2.1. O Lúpus Eritematoso Sistêmico (LES)

O LES, é uma doença inflamatória multiorgânica que compromete cerca de cinco milhões de pessoas no mundo. É o protótipo da doença autoimune sistêmica, sua etiologia é desconhecida, e sua característica marcante é o grande pleomorfismo clínico e consequentemente fisiopatogênico (Rivas-Larrauri and Yamazaki-Nakashimada 2016).

2.1.1 Imunopatogênese

O LES é uma doença inflamatória sistêmica, multiorgânica, que tem como seu pilar fisiopatogênico a resposta imune direcionada ao próprio (autoimunidade). Sua etiologia é desconhecida ainda hoje, e seu paradigma fisiopatogênico atual defende, que um indivíduo geneticamente predisposto, ao entrar em contato com os diversos estímulos ambientais (biológicos, físicos e/ou químicos), desenvolve uma quebra da tolerância imunológica e deflagra uma

resposta imune ao próprio, gerando inflamação e dano tecidual (Parks, de Souza Espindola Santos et al. 2017) (**Figura 1**).

A variedade de fenótipos clínicos do LES, demonstra, por si só, a complexidade dos mecanismos envolvidos em sua indução, amplificação e manutenção. Este processo culmina com intensa produção de autoanticorpos dirigidos da diversos epítopos endógenos e aumento nos níveis de interferon (IFN) tipo 1 e do sistema *B-cell activating factor (BAFF)/B lymphocyte stimulator (BlyS)*, características marcantes da doença (Blanco, Palucka et al. 2001, Petri, Singh et al. 2009, Salazar-Camarena, Ortiz-Lazareno et al. 2016, Steri, Orrù et al. 2017).

Apesar do LES poder resultar da deficiência de apenas um gene (i.e. C1q, TREX1, DNASE1L3), na maioria dos casos, a herança é poligênica, podendo ocorrer interação entre dois ou mais *loci*, de forma a aumentar aditivamente, o risco de desenvolver a doença (Costa-Reis and Sullivan 2017). Vários *loci* estão associados a um maior risco de LES; entre eles estão HLA, genes que codificam proteínas relacionadas à degradação de DNA, apoptose, *clearance* de debris celulares, detecção de ácidos nucléicos e produção de IFN tipo 1 por células dendríticas (DCs), diminuição do limiar de ativação de linfócitos T e B, entre outras, conforme mostra a **Tabela 1** (Deng and Tsao 2014, Bentham, Morris et al. 2015, Morris, Sheng et al. 2016). Isto demonstra o envolvimento dos sistemas inato e adaptativo na imunopatogênese da doença, e explica porque a imunossupressão ampla e inespecífica (feita com glicocorticóides e outros imunossupressores sintéticos), costuma ser mais eficaz que medicamentos biológicos direcionados a apenas um alvo molecular, sendo ainda a primeira linha terapêutica no LES (Bertsias, Ioannidis et al. 2008).

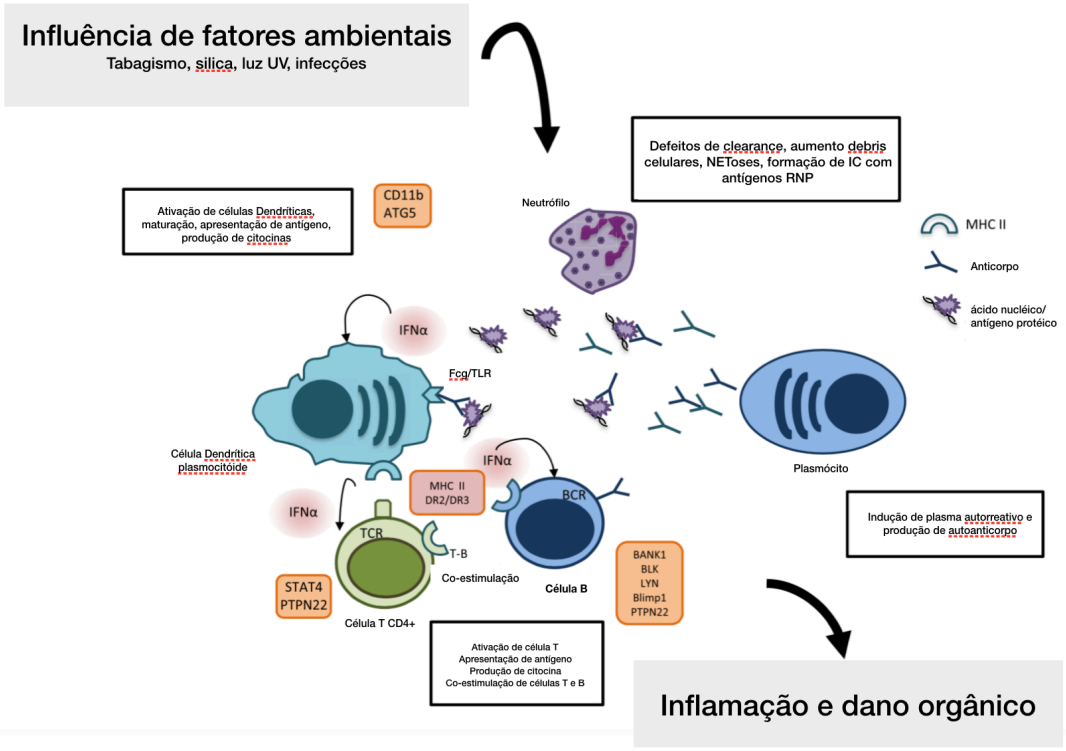

Figura 1. Imunopatogênese do LES. Um indivíduo geneticamente predisposto, entra em contato, ao longo da vida, com determinados fatores ambientais capazes de induzir a ativação imune, quebra da tolerância e desenvolvimento de uma resposta contra antígenos próprios. Diversas alterações contribuem para este mecanismo: aumento da quantidade de restos celulares circulantes (proveniente de apoptose, necrose, NETose, etc), clearance defeituoso destes debris celulares, ativação de DCs e apresentação destes antígenos próprios, ativação de clones de linfócitos T e B autorreativos, produção de autoanticorpos, e formação de imunocomplexos. Todos estes eventos contribuem para a instalação de inflamação sistêmica e tecidual, com dano orgânico que culminam com as manifestações clínicas da doença. Adaptada de (Deng and Tsao 2014)

Dentre os fatores ambientais, vários agentes infecciosos parecem ter associação com a doença. Entre eles estão bactérias (Doaty, Agrawal et al. 2016), fungos (Doaty, Agrawal et al. 2016) e vários vírus como Citomegalovírus (CMV) (Sekigawa, Nawata et al. 2002), Epstein Barr vírus (EBV) (Ascherio and Munger 2015), Parvovírus B19 (PVB19) (Aslanidis, Pyrpasopoulou et al. 2008), Herpes simplex vírus (HSV), Varicela zoster vírus (VZV) (Ramos-Casals, Cuadrado et al. 2008) entre outros. O desenvolvimento do LES não pôde, até o momento, ser ligado a uma única infecção prévia. O mais provável, é que o

encontro seqüencial com os diversos patógenos, ao longo da vida de um indivíduo geneticamente predisposto, estimule receptores *Toll like* (TLRs) e sensores citosólicos, determinando uma resposta por parte das células apresentadoras de antígenos (APCs), com subsequente resposta T (Th1, Th2, Th17 e/ou Treg) alterada. Um dos mecanismos cogitados como contribuidor da quebra de tolerância, é o mimetismo molecular, no qual seqüências peptídicas de antígenos nos patógenos são similares a epítopos de estruturas próprias, gerando a ativação de clones auto-reativos (Aas-Hanssen, Thompson et al. 2015). Além disso, devido à oferta aumentada de debris celulares (provenientes de apoptose, NETose e necrose aumentadas), ao *clearance* ineficaz destes restos, e ao ambiente citocínico pró-inflamatório determinado pela infecção; ocorre a apresentação de antígenos próprios pelas APCs e seu reconhecimento por linfócitos T auto-reativos, levando à sua ativação e ao estabelecimento de uma resposta imune adaptativa direcionada ao próprio (Mahajan, Herrmann et al. 2016).

Outros fatores ambientais, como agentes físicos (ex. luz UV) e químicos (ex. fármacos, tabagismo), influenciam a doença, por meio da indução de morte celular, da modificação de proteínas e de mecanismos epigenéticos, como a metilação do DNA e a modificação pós-translacional de histonas (Barbhaiya and Costenbader 2016). O *status* metabólico do indivíduo, sua dieta, sua microbiota e as características metabólicas intrínsecas das células de seu sistema imune, também parecem influenciar profundamente a sua resposta imunológica.

Diversas anormalidades imunometabólicas já foram evidenciadas em modelos experimentais de LES e em seres humanos com a doença (Romo-Tena and Kaplan 2020). No LES, as células mais estudadas, quanto ao

imunometabolismo, são os linfócitos T. As diversas alterações descritas como prováveis influenciadoras das funções, fenótipos e sobrevida destas células, incluem anormalidades mitocondriais (Gergely, Grossman et al. 2002), estresse oxidativo (Perl, Hanczko et al. 2015), ativação aumentada do sensor metabólico mTORC1 (Lui, Tsang et al. 2008, Fernandez and Perl 2010), *up regulation* do receptor de glicose GLUT6 (Li, Wu et al. 2012), hiperativação da via da Pentose Fosfato (PPP) (Lui, Tsang et al. 2008), e alterações no metabolismo lipídico (Kidani and Bensinger 2014). Outras populações celulares como linfócitos B (Caro-Maldonado, Wang et al. 2014, Lam, Becker et al. 2016), macrófagos, neutrófilos (Mohammadi, Saghaeian-Jazi et al. 2017) e células dendríticas (DCs) (Westerterp, Gautier et al. 2017) também têm demonstrado alterações em sua programação metabólica no LES.

As múltiplas interações entre os vários genes implicados, as modificações epigenéticas e os estímulos ambientais resultam nesta doença, que chamamos LES, e que, na verdade, é consequência de um amplo desajuste da resposta imune, com múltiplos epítopos, células, moléculas e vias metabólicas e de sinalização envolvidos. Isto resulta em uma doença com quadro clínico diversificado, o que resulta em uma dificuldade clínica no diagnóstico, tratamento e acompanhamento.

Tabela 1. Principais genes associados ao desenvolvimento do Lúpus Eritematoso Sistêmico

Via	Cromossomo	Gene
Sinalização IFN tipo I	2q24	IFIH1
	2q32	STAT4
	5q34	miR146a
	7q32	IRF5
	11p15	IRF7
	12q24.32	SLC15A4
	16q24	IRF8
	19p13	TYK2
	Xp22	TLR7
Sinalização NFkB	5q33.1	TNIP1
	6q23	TNFAIP3
	22q11.21	UBE2L3
	Xq28	IRAK1/MECP2
Sinalização de células B e T	1p13.2	PTPN22
	1q25	TNFSF4
	1q31q32	IL10
	2p25-p24	RASGRP3
	3q13	CD80
	4q21	AFF1
	4q24	BANK1
	4q26-q27	IL21
	6q21	PRDM1
Função de Neutrófilos e monócitos	19p13	ICAM1/4/5
***Clearance* de imunocomplexos**	1q23	FCGR2A/FCGR2B/FCGR3A/FCGR3B
	16p11.2	ITGAM
Via NADPH oxidase dependente	1q25	NCF2
Outros	3q13.33	TMEM39A
	5q35	PTTG1
	12p13	CDKN1B

2.1.2 Epidemiologia

O LES é uma doença cosmopolita que acomete cerca de 1,5 milhão de pessoas somente nos Estados Unidos da América. Sua incidência e prevalência variam de acordo com a população estudada, o país e a metodologia empregada; mas em geral encontram-se entre 1,0 a 8,7 casos/100.000 e 5,8 a 130 casos/100.000 pessoas respectivamente (Siegel and Lee 1973, Uramoto, Michet et al. 1999, Vilar and Sato 2002). Há, contudo, locais onde pode alcançar uma incidência de 63,7/100.000/ano e prevalência de 1000/100.000, como em Gainesville, Georgia (EUA) (Kardestuncer and Frumkin 1997).

A faixa etária mais acometida é o adulto jovem, e a idade média no início da doença em torno de 33 anos(Sassi, Hendler et al. 2017) com tendência a ser mais precoce em negros e em mulheres (Yen, Shaheen et al. 2017). Apesar de poder atingir ambos os sexos, o número de casos é maior no sexo feminino, chegando a até 13 casos em mulheres para 1 em homens, nesta faixa etária, e caindo expressivamente, entre crianças (1,4 a 5,8:1) e idosos (2:1) (Sassi, Hendler et al. 2017).

2.1.3 Quadro clínico

O LES pode envolver, virtualmente, qualquer sistema orgânico. Isto torna o seu quadro clínico bastante pleomórfico, não apenas nos sinais e sintomas, mas também na gravidade, evolução e resposta ao tratamento. Assim, o diagnóstico não se baseia apenas em um parâmetro, mas é feito por meio da avaliação conjunta de aspectos clínicos, laboratoriais e, por vezes, histopatológicos nos indivíduos acometidos, o que, por vezes o retarda (Thong and Olsen 2017).

As primeiras manifestações são, geralmente, inespecíficas e incluem febre, astenia, mal-estar geral, hiporexia e perda ponderal. Com a evolução da doença, outras alterações surgem (na dependência do aparelho afetado), se estabelecendo, na maioria dos casos, um quadro clínico inflamatório e multiorgânico(Thong and Olsen 2017).

As manifestações clínicas mais comuns são artrite (80%), lesões cutâneas (71%), disfunção cognitiva (50%) e leucopenia (46%). Contudo, qualquer sistema orgânico pode ser afetado pela doença (Fortuna and Brennan 2013). Os comprometimentos considerados mais graves são os decorrentes do envolvimento do sistema nervoso central (SNC), pulmões, coração e rins, sendo o comprometimento renal responsável por atingir 35 a 75% dos pacientes em algum momento no curso da doença, a maioria nos três primeiros anos após o diagnóstico (Maroz and Segal 2013).

2.1.4 O Lúpus e o rim

As manifestações renais do LES são denominadas nefrite lúpica e compreendem um grupo heterogêneo de alterações. O comprometimento pode atingir glomérulos, túbulos, interstício e vasos sanguíneos em combinações e intensidades variáveis (Rovin, Parikh et al. 2014).

O mecanismo imunopatogênico principal é a reação de hipersensibilidade tipo III de Gell e Coombs, com uma superprodução de auto-anticorpos direcionados a antígenos nucleares, formação de imunocomplexos e sua deposição. Nos rins, essa deposição pode ocorrer em qualquer um de seus compartimentos — glomérulos, membranas basais dos capilares tubulares e peritubulares ou vasos maiores (Lewis and Schwartz 2005). Os fatores que

governam o local da deposição destes imunocomplexos, provavelmente têm relação com fatores hemodinâmicos peculiares ao próprio glomérulo, assim como com as propriedades fisicoquímicas dos imunocomplexos, incluindo sua quantidade, especificidade, avidez, tamanho, carga elétrica e isotipo.

Os imunocomplexos podem ser pré-formados ou podem se formar localmente no rim. Antígenos circulantes como nucleossomos carregados positivamente, podem se fixar em sítios aniônicos, por meio da interação de cargas, na parede dos capilares glomerulares e, uma vez fixados, estes autoantígenos interagem com autoanticorpos circulantes levando à formação *in situ* de imunocomplexos (Foster, Cizman et al. 1993, Berden, Licht et al. 1999). O depósito imune no rim é abundante e diverso quando analisado por imunofluorescência, sendo composto de IgG, IgM e IgA (padrão *full house*), além de depósitos de C1, C3 e properdina, o que denota a ativação do complemento e recrutamento de diversas células efetoras, com conseqüente lesão tecidual.

A Organização Mundial de Saúde (OMS), em 1982, classificou a nefrite lúpica em seis subtipos distintos, na dependência de suas características histopatológicas (**Tabela 1**) (Weening, D'Agati et al. 2004); e em 2003, esta classificação foi revisada e modificada pela *International Society of Nephrology/Renal Pathology Society* (ISN/RPS) (**Tabela 2**). O *National Institutes of Health* (NIH) elaborou índices de atividade e cronicidade para a nefrite, com base nos achados histopatológicos, e estes são utilizados de forma complementar à classificação da nefrite. As características histopatológicas da nefrite referentes a sua classificação e índices de atividade e cronicidade, se correlacionam com a gravidade clínica, o prognóstico e orientam o tratamento específico e de suporte.

Tabela 2. Classificação histopatológica da nefrite lúpica pela Organização Mundial de Saúde (OMS) 2004.

Classe	Descrição
Classe I	Glomerulonefrite mesangial mínima
Classe II	Glomerulonefrite mesangial proliferativa
Classe III	Glomerulonefrite focal
Classe IV	GLomerulonefrite difusa segmentar ou difusa global
Classe V	Glomerulonefrite membranosa
Classe VI	Esclerose glomerular avançada

O diagnóstico da nefrite é feito, preferencialmente, pela avaliação histopatológica do fragmento de rim retirado por biópsia percutânea. Todo paciente com suspeita de comprometimento renal pelo LES, deve ser submetido à biópsia renal para sua classificação histopatológica e avaliação de atividade e cronicidade. Em situações nas quais a biópsia não está disponível, o diagnóstico e a classificação da nefrite podem ser feitos com base nos achados clínicos e laboratoriais. Estes achados não são suficientes para determinar quais compartimentos renais estão comprometidos, quais as características deste comprometimento, ou qual a classe exata da nefrite. Contudo, na maioria das ocasiões, eles demonstram claramente se há ou não nefrite, e se ela apresenta sinais de gravidade.

A definição de caso de nefrite lúpica pelo *American College of Rheumatology* (ACR) se estabelece pelos seguintes achados clínicos e laboratoriais: proteinúria persistente >0,5 g nas 24 horas ou maior que 3+ pelo *dipstick*, e/ou sedimento urinário ativo (>5 hemácias (RBCs) por campo, >5

leucócitos (WBCs) por campo, na ausência de infecção, ou cilindros celulares incluindo: RBCs, hemoglobina, granulares, tubulares ou mistos (Hahn, McMahon et al. 2012). A relação proteína/creatinina >0,5 em amostra isolada de urina, pode substituir a proteinúria de 24 horas.

O tratamento específico destes pacientes é feito com drogas imunossupressoras escolhidas de acordo com a classe histopatológica e o perfil clínico de cada paciente. O tratamento de suporte se baseia, principalmente em drogas antiproteinúricas, estatinas e medidas de estilo de vida como cessação de tabagismo, restrição no consumo de sódio, entre outras (Wilhelmus, Bajema et al. 2016). Mesmo assim, uma parte dos pacientes não alcança a remissão total e 10% de todos os indivíduos que desenvolvem nefrite lúpica, evoluem para doença renal terminal, com necessidade de terapia substitutiva e transplante. Esse risco é maior quando se leva em conta as formas mais graves de nefrite, como a classe IV (proliferativa difusa), a qual confere um risco de 44% ao longo de 15 anos e uma mortalidade maior do que os pacientes sem comprometimento renal pela doença (Quintana and Jayne 2016). O diagnóstico precoce e o monitoramento eficaz (capaz de detectar recaídas de forma sensível ou mesmo prevê-las com antecedência) é de grande importância e teriam um impacto positivo na evolução e prognóstico destes pacientes.

2.1.5 Monitoramento da nefrite lúpica

O monitoramento dos pacientes com nefrite é feito utilizando-se dados clínicos e laboratoriais. Dentre os exames complementares normalmente empregados estão a creatinina sérica, sedimento urinário (leucocitária,

hematúria, cilindrúria celular) e a proteinúria, os quais, quando alterados, podem demandar a repetição de uma biópsia renal.

Tabela 3. Classificação da Nefrite Lúpica pela *International Society of Nephrology/Renal Pathology Society* (ISN/RPS) 2003

Classe	Descrição
I	Mínima
II	Mesangial
III	Membranoproliferativa focal
(A)	Lesões ativas
(A/C)	Lesões ativas e crônicas
(C)	Lesões crônicas
IV	Membranoproliferativa difusa
S(A)	Segmentar/atividade
G(A)	Global/atividade
S(A/C)	Segmentar/Lesões ativas e crônicas
G(A/C)	Global/Lesões ativas e crônicas
S(C)	Segmentar/Crônica
G(C)	Global/Crônica
V	Membranosa
	+ III ou IV
	Esclerose avançada
VI	90% esclerose global sem atividade residual

As diretrizes do *Joint European League Against Rheumatism and European Renal Association - European Dialysis and transplant Association (EULAR/ERA-EDTA)* para o manejo da nefrite lúpica (NL) (Fanouriakis, Kostopoulou et al. 2019), recomenda que seja avaliado em cada consulta o peso corporal, pressão arterial, creatinina sérica (com o cálculo da taxa de filtração glomerular - TGF), albumina sérica, proteinúria, sedimento urinário, C3 e C4 séricos, anti-DNAds e hemograma. A cinética da proteinúria e da creatinina

sérica nos primeiros 12 meses são mais sensíveis que a hematúria na predição do prognóstico a longo prazo. Estudos tem demonstrado que a proteinúria ao fim do primeiro ano de tratamento (< 0,8g nas 24h) foi o melhor preditor de desfecho renal (Medina-Rosas, Yap et al. 2016, Medina-Rosas, Fung et al. 2018).

Apesar de serem amplamente utilizados no mundo inteiro, os parâmetros clínicos não são suficientes, e os testes convencionais empregados são de sensibilidade e especificidade limitadas. Esdaile et al. avaliaram a acurácia diagnóstica para predição de recaídas renais do anti-DNAds, C3, C4 e anti-C1q em 202 pacientes com LES, e chegaram a conclusão de que eram pobres preditores de exacerbação da doença (Esdaile, Joseph et al. 1996). Moroni et al. avaliaram acurácia diagnóstica para detecção de nefrite ativa vigente, e observaram diferença na sensibilidade do anti-DNAds e C3/C4 quando foi levada em consideração a classificação histopatológica da nefrite, com a sensibilidade mais alta nas formas proliferativas da doença (70% e 79% respectivamente) (Moroni, Radice et al. 2009).

Em uma revisão sistemática de 69 estudos avaliando a acurácia diagnóstica do anti-DNAds, C3 e C4, foram encontrados resultados heterogêneos, mostrando uma performance limitada destes exames e dependente do seu grau de anormalidade e da probabilidade pré teste de nefrite ativa, conforme demonstrado na **Tabela 4** (Gensous, Marti et al. 2017).

O padrão-ouro para o diagnóstico de NL continua sendo a biópsia renal com a análise histopatológica do material obtido (Almaani, Meara et al. 2017). A histopatologia do rim permite: (a) a estratificação da NL de acordo com a classificação da Organização mundial de saúde (OMS) modificada pela *Renal*

Pathology Society/International Society of Nephrology Working Group on the classification of lupus nephritis (RSP/ISN 2003); (b) a avaliação da presença de lesão inflamatória ativa e/ou seqüelar (índices de atividade e cronicidade do *National Institutes of Health - NIH*); (c) a verificação da presença de comprometimento em outros compartimentos renais — como o vascular e o tubulointersticial; (d) e a identificação de outros tipos de lesões sejam elas autoimunes ou não (e.g. nefropatia por IgA, nefropatia diabética, hipertensiva, etc) (Bajema, Wilhelmus et al. 2018).

Tabela 4. Acurácia diagnóstica do anti-DNAds, C3 e C4 para predição de recaídas.

Classe	anti-DNAds (alta concentração)	C3 diminuído	C4 diminuído
Sensibilidade (%)	26 - 100	29 - 51	19 - 53
Especificidade (%)	13 - 89	63 - 88	74 - 79
Valor preditivo positivo (%)	4 - 59	2 -12	3 - 7
Valor preditivo negativo (%)	67 - 97	95 -98	96 - 98

Apesar de seus benefícios, a biópsia não está disponível em todos os serviços de saúde, necessita de uma estrutura e treinamento mínimos para ser realizada e carreia em si mesma, risco de complicações (e.g. perda do rim biopsiado, morte, entre outros) (Bandari, Fuller et al. 2016). Por conta disto, torna-se inviável a realização freqüente da biópsia, apenas para monitoramento da terapêutica, ponderando-se riscos e benefícios para tomar a decisão de efetuá-la. O limiar clínico para a sua execução varia entre serviços e diretrizes, sendo usualmente feita para o diagnóstico inicial, quando há curso clínico atípico, refratariedade ao tratamento ou recidiva (Wilhelmus, Bajema et al. 2016). Por

esta razão o número de pesquisas buscando novos e melhores biomarcadores tem sido crescente nas últimas décadas.

Biomarcadores (BM) são características biológicas que indicam um fenômeno biológico normal, patogênico, a presença de uma intervenção ou a resposta a ela. O biomarcador ideal para a NL deveria ser capaz de (a) identificar os indivíduos com risco de desenvolver NL, (b) determinar o seu risco de progressão, (c) distinguir entre alterações decorrentes de atividade inflamatória ou sequelas, (d) estratificar a escolha e a duração do tratamento, (e) ter um tempo de resposta curto para auxiliar decisões terapêuticas, (f) ser mais sensível e específico que os BM convencionais, (g) ser de fácil execução, (h) ter boa reprodutibilidade, (i) não ser caro, e (j) ter um impacto positivo em desfechos clínicos relevantes, e.g., progressão para doença renal terminal (DRT), qualidade de vida e mortalidade causa-específica (Aronson and Ferner 2017, Capecchi, Puxeddu et al. 2020).

O surgimento de um BM passa por diversas etapas, desde a identificação de uma característica relevante dentro da patogênese da doença em questão, sua plausibilidade como possível biomarcador em estudos pré-clínicos, e a testagem de sua performance diagnóstica em diferentes delineamentos de pesquisa subsequentes.

Dentre os diversos biomarcadores que vem sendo estudados na NL, estão BM genéticos (Lu, Kwan et al. 2011, Wither, Prokopec et al. 2018, Yang and Li 2019, Aguirre-Valencia, Rios-Serna et al. 2020), anticorpos (Zhang, Pan et al. 2010, Ben-Ami Shor, Blank et al. 2014, Metwally, Eesa et al. 2019), citocinas (Jakiela, Kosałka et al. 2018, Selvaraja, Abdullah et al. 2019), quimiocinas (Jakiela, Kosałka et al. 2018), moléculas de adesão (Daniel, Sichez

et al. 2001, Nakatani, Fujii et al. 2004, Skeoch, Haque et al. 2014), fatores de crescimento (Mohammed, Mok et al. 2003, Resende, Elias et al. 2017), moléculas da superfície celular (Bellan, Quaglia et al. 2021), populações e subpopulações celulares (Liu, Li et al. 2020, Yap, Yung et al. 2020), etc.

Uma revisão sistemática (RS) feita por Radin et al. revelou que existem mais de 70 novos biomarcadores urinários e séricos estudados na NL em mais de 80 estudos diferentes (Radin, Miraglia et al. 2021). Algumas revisões sistemáticas (RS) avaliaram individualmente novos biomarcadores séricos e urinários testados em pacientes com nefrite lúpica, como o *Neutrophil gelatinase-associated lipocalin* (NGAL) (Gao, Wang et al. 2020), *Monocyte chemoattractant protein 1* (MCP-1) (Lee and Song 2017) e *Interferon-inducible protein 10* (IP-10) (Puapatanakul, Chansritrakul et al. 2019), entre outras. Em uma pesquisa preliminar na base de dados Pubmed, apesar de diversas revisões narrativas abordarem o assunto, não foram encontradas *Overviews.*

A realização de uma *Overview* das revisões sistemáticas sobre os novos biomarcadores na nefrite lúpica, proporciona a compilação das evidências disponíveis sobre o assunto e uma compreensão maior sobre o estado da arte neste tema. Somado a isto, permite a sumarização, detalhamento, avaliação e interpretação do que estes biomarcadores significam em termos de aplicabilidade na prática clínica; determinação da necessidade de novas pesquisas sobre os biomarcadores já estudados e direcionamento de novos caminhos de pesquisa, de forma sistemática, objetiva e fundamentada.

O objetivo deste trabalho foi realizar uma *Overview* de revisões sistemáticas sobre acurácia diagnóstica dos novos biomarcadores estudados na Nefrite Lúpica.

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Título: “Diagnostic test accuracy of novel biomarkers for lupus nephritis - An overview of systematic reviews”

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with multiorgan inflammatory involvement. The mortality rate for individuals with SLE is 2.6-fold higher than that the same age and sex in the general population (1). Approximately 50% of patients with SLE develop renal impairment, i.e., lupus nephritis (LN) (2-4). LN consists of renal alterations that can compromise the glomerulus, interstitium, tubules, and blood vessels, with different severities and combinations (2). The great importance of LN lies in the significant number of affected patients and the potential to directly influence patient prognosis (5, 6).

The mortality associated with SLE is higher in patients with LN than in those without renal impairment. If only patients with severe proliferative forms of the disease (class III and IV) are considered, the mortality rate is as high as 25% (7, 8).

Treatment for LN has drastically changed patient survival in recent years. However, 10 to 30% of patients still progress to end-stage renal disease and require dialysis and transplantation (9). Despite its potential severity, the initial clinical manifestations of LN are often discrete or absent and are usually detected through complementary tests (10).

The gold standard for diagnosing LN is the histopathological analysis of renal tissue obtained by percutaneous biopsy. Kidney histopathology allows (a) the stratification of LN based on the World Health Organization (WHO) classification modified by the Renal Pathology Society/International Society of Nephrology Working Group on the Classification of Lupus Nephritis (RSP/ISN 2003) (11, 12); (b) the

evaluation of the presence of active and chronic inflammatory lesions (activity and chronicity indices of the National Institutes of Health - NIH) (13); (c) the verification of the presence of disease in other renal compartments - such as the vascular and tubulointerstitial compartments; (d) and the identification of other coexistent lesions, whether autoimmune or not (e.g., IgA nephropathy, diabetic nephropathy, hypertensive nephropathy, etc.).

However, the biopsy is not widely available in all health services; it requires infrastructure, training and carries the risk of complications, such as hematuria, loss of the biopsied kidney, or even death (14). Therefore, biopsies are not performed for frequent routine monitoring, and the decision to perform a biopsy on a patient involves weighing risks and benefits. Monitoring renal involvement in SLE is achieved using the following serum and urinary biomarkers: anti-DNAds, serum complement levels (C3 and C4), creatinine clearance, urinalysis with urine sediment microscopy, and proteinuria, represented by 24-hour proteinuria or protein/creatinine ratio in an isolated urine sample (15, 16). These are considered the traditional biomarkers and are adopted by the international guidelines for lupus nephritis management.

Although they have been used in clinical practice for several years, their use has some limitations. Their accuracy is limited (7, 17), compromising the distinction between active and chronic renal lesions and the differentiation between LN and comorbidities that may be concomitant in an individual SLE patient. In addition, studies that have evaluated the possible advantages of performing programmed repetition of biopsies showed clinicopathological dissociation. Malvar et al. observed that one-third of patients who had achieved clinical remission of nephritis had active inflammatory lesions in the histopathological analysis of the kidney and that 62% of individuals considered to have active kidney disease were in histopathological remission (18).

Delayed diagnosis of LN is associated with a higher risk of progression to end-stage renal disease, the need for replacement therapy, and mortality (19). Thus, improving the prognosis of these patients involves early detection of the disease, definition of its severity, and prediction of its response to treatment and relapse.

In recent years, a great scientific effort has been exerted in the search for new, more sensitive, and specific biomarkers. Several studies suggest possible candidates, such as genes (20-23), antibodies (24-26), cytokines (27, 28), chemokines (27), adhesion molecules (29-31), growth factors (32, 33), cell surface molecules (34), and cell populations (35, 36), among others.

Some systematic reviews (SRs) have individually evaluated new serum and urinary biomarkers tested in patients with LN, for example, neutrophil gelatinase-associated lipocalin (NGAL) (37), monocyte chemoattractant protein 1 (MCP-1) (38), and interferon-inducible protein 10 (IP-10) (39). In a preliminary search in the PubMed database, although several narrative reviews address this subject, no overviews were found.

This study aimed to summarize SRs on the accuracy of novel serum and urinary biomarkers for diagnosing LN in patients with SLE. The research question is "*What is the accuracy of the novel serum and urinary biomarkers studied for the diagnosis of LN in patients with SLE?".*

Methods

Protocol and registration

The protocol for this overview was registered in August 2020 on the Prospero platform of the University of York under the number **CRD42020196693**.

Selection criteria

Type of studies

Systematic Reviews (SR), with or without meta-analyses, of observational studies evaluating the diagnostic accuracy of serum or urinary new biomarkers of LN were included.

Participants

Participants in the included studies were patients diagnosed with SLE, classified by the ACR (1997), SLICC (2012), or ACR/EULAR 2019 criteria, in the outpatient or in-hospital settings, without sex or age restrictions.

Index test

Studies evaluating new serum and urinary biomarkers, or combinations of these biomarkers (biomarker panels) tested for the detection of LN were included.

Reference test

Currently, the reference tests used in clinical practice include anti-DNAds, C3, C4, creatinine clearance, urinalysis with sediment microscopy, 24-h proteinuria or protein/creatinine ratio in an isolated urine sample, and renal biopsy. These biomarkers are considered standard by the *European Alliance of Associations for Rheumatology* (EULAR) and the *American College of Rheumatology* (ACR). They are widely used for the detection and monitoring of LN.

Primary studies evaluating the diagnostic accuracy of LN biomarkers usually use a combination of tests to define the presence of nephritis. Given this peculiarity of this field of research, this overview considered all SRs of studies that evaluated new

biomarkers by comparing patients with and without LN, patients with active and inactive LN, patients with renal relapse, and without renal relapse, and patients with proliferative and non-proliferative LN using any combination of those tests.

Outcome measures

The primary outcome was the diagnostic accuracy of each biomarker to identify LN in patients with SLE. The secondary outcomes of interest were the diagnostic accuracy for detecting active LN, prediction of renal relapse, identification of response to treatment, and differentiation between proliferative and nonproliferative LN forms.

Exclusion criteria

SRs evaluating biomarkers for detecting only other clinical manifestations of disease activity in SLE; those that did not describe the quantitative data relative to diagnostic accuracy of the test assessing the biomarkers; and those evaluating only genetic biomarkers (search for genes and variants), imaging and histopathological techniques were excluded. Primary studies, case reports, narrative reviews, and other types of publications, such as editorials, comments, and letters were excluded as well.

Literature search

The databases used to search for evidence were PubMed, EMBASE, BIREME/LILACS, Scopus, Web of Science, and Cochrane, including gray literature found through Google Scholar and PROQUEST, from inception until April 2022. The search strategy was developed based on the PIRD (Population, Index test, Reference test, Diagnosis) approach with an information specialist (KL), using free-text and subject headings referring to SLE, LN, and biomarkers. The type of study was not included in the search strategy to increase its sensitivity. **S1 Table** provides the search strategy

constructed for all databases searched. This strategy was adapted to the other databases. No language restriction was applied.

Selection of studies

The selection of studies was performed by two reviewers (JARG and BM) after the removal of duplicates using EndNoteX9. This process was done in two stages. In the first stage, studies were selected based on titles and abstracts, and in the second stage, studies were selected based on full text analysis, checking the eligibility criteria. Disagreements were resolved by consensus and, in case of persistent discrepancies, the decision was made by a third reviewer (FM).

Data extraction and management

Data were extracted by two authors (JARG and BM), into a table containing the following information: review question, objectives, population (characteristics, total number), clinical context (outpatient, hospital), index biomarker, reference biomarker, biological material, technique used, details of the search, outcome, databases searched, date range of included studies, number of included studies, methodological quality assessment tool, diagnostic accuracy results, heterogeneity, publication bias, and conclusion.

Data analysis

Extracted data were analyzed by three reviewers (JARG, JFMB, and SCF), qualitatively summarized, and presented in tables. Data from selected SRs were reported as diagnostic accuracy measures: pooled sensitivity, pooled specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and summary ROC curve area under the curve (SROC-AUC).

It was reported when more than one SR evaluating the same biomarker presented similar conclusions. When conflicting results existed, the possible reasons were explored.

Assessment of reporting bias

The Deeks test was used to investigate possible publication bias, if possible. Despite the limitations of the evaluation of this aspect in systematic reviews of diagnostic tests accuracy, the likelihood of publication bias was reduced by the extensive search of studies in the databases already cited, in the gray literature, hand searching the references, and by including conference proceedings.

Assessment of methodological quality

The risk of bias of the included reviews was analyzed by two reviewers (JARG e BM) using the ROBIS tool (40). Any disagreements were judge by a third author (ACSL).

Results

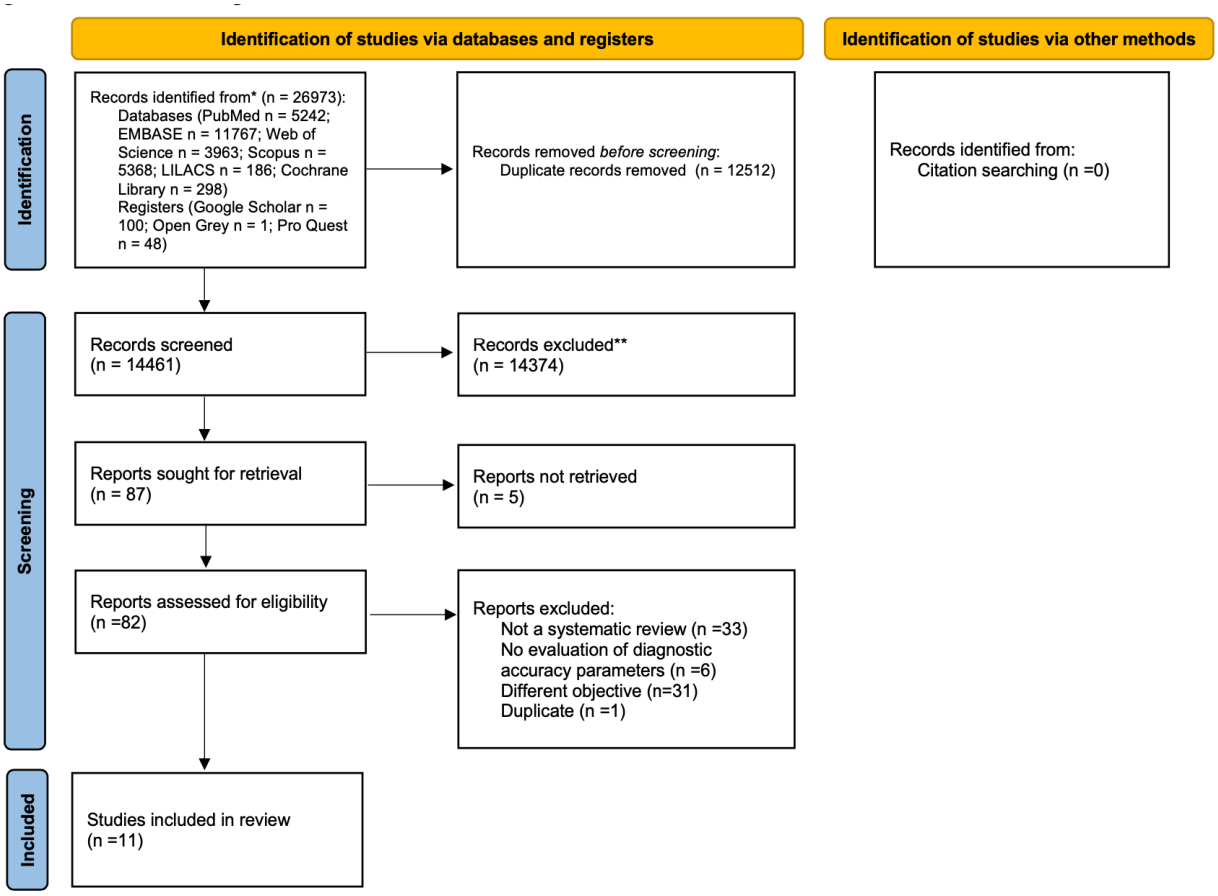
In total, 26,973 articles addressing biomarkers (BMs) in lupus nephritis (LN) were identified. After exportation to EndNote, 12,512 duplicates were detected and removed. During Phase 1, 14,461 articles were evaluated by titles and abstracts, leaving 87 articles for full-text analysis. Finally, 11 systematic reviews (SRs) met the eligibility criteria and were included in this overview, as shown in **Fig 1**.

Description of the included reviews

Eleven SRs on the diagnostic accuracy of new serum and urinary BMs in LN were selected. The SRs evaluated 7 distinct BMs in a total of 21 review arms (10 that analysed serum BMs (39, 41-44, 74,127), and 13 that analysed BMs in urine (37, 39, 45-47,74, 127), as shown in **Table 1**. The population evaluated in the primary studies was

predominantly adults. Two SRs included strictly adults, 6 reviews also included studies in the paediatric population, and 4 did not report the age groups (**Table 2**).

Fig 1. Overview flow-diagram.



The SRs included observational cross-sectional studies (85 studies), cohort studies (36 studies), 4 case-control studies, 4 longitudinal and cross-sectional and 1 longitudinal study. Seven SRs (12 arms) did not report the designs of the included primary studies. The main outcomes evaluated were the accuracy of the BM for the diagnosis of LN in patients with systemic lupus erythematosus (SLE) (37, 39, 41-46, 74), the detection of disease activity (37, 42, 44-47, 74), the prediction of renal relapse (37, 45) and the stratification of severity (37). No review evaluated prognosis or early response to treatment. The main characteristics and data of the included SRs are shown in **Table 1** and **Table 2**.

Table 1. Overview of key characteristics of included reviews.

author (year)	Country	Search date	Population	Index test	Biological sample	Tipo de BM	Reference test	Diagnosis	Meta-analysis	Number of included studies	Study design
Benito-Garcia, E et al. (2004)	USA	January 1966 - December 2003	SLE patients	anti-Sm	serum	antibody	Renal biopsy; clinical parameters	LN	No	13 (8 metanalysed)	NI
			SLE patients	anti-RNP	serum	antibody	Renal biopsy; clinical parameters	LN	No	8	NI
Yin, Y. et al. (2012)	China	Until October 2011	SLE patients	anti-C1q	serum	antibody	Renal biopsy; clinical parameters	LN	Yes	7	NI
			LN patients	anti-C1q	serum	antibody	Renal biopsy; clinical parameters	LN activity	Yes	22	NI
Eggleton P. et al. (2014)	United Kingdom	1977 - 2013	SLE patients	anti-C1q (ELISA)	serum	antibody	Renal biopsy; clinical parameters	LN	Yes	25 (22 meta-analysed)	NI
			LN patients	anti-C1q (ELISA)	serum	antibody	Renal biopsy; clinical parameters	LN activity	Yes	31 total (28 meta-analysed)	NI
Fang Y. G. et al. (2015)	China	Until December 2014	SLE patients	uNGAL	urine	acute phase glycoprotein	Renal biopsy	LN	Yes	4	CS
			LN patients	uNGAL	urine	acute phase glycoprotein	SLEDAI; SLICC; BILAG-2004; SLEDAI 2000;	LN activity	Yes	8	3 CS; 5 PC
			LN patients	uNGAL	urine	acute phase glycoprotein	SLEDAI; SLEDAI 2000; BILAG-2004; clinical parameters	Prediction of LN flares	Yes	6	1 CS; 5 PC
Wang, Z. et al. (2015)	China	Until March 2015	SLE patients	anti-C1q	serum	antibody	Renal biopsy; clinical parameters	LN	Yes	3	NI
Puapatanakul, P; et al. (2019)	Tailândia	Until December 2017	SLE patients	IP-10	serum	chemokine	SLEDAI; BILAG; SLAM-R renal biopsy; SELENA-SLEDAI; clinical parameters	LN	No	2	NI
			SLE patients	IP-10	urine	chemokine	SLEDAI; BILAG; SLAM-R renal biopsy; SELENA-SLEDAI; clinical parameters	LN	No	5	NI
Gao, Y; et al. (2020)	China	Until October 2019	SLE patients	uNGAL	urine	acute phase glycoprotein	Renal biopsy	LN	Yes	6	PC
			LN patients	uNGAL	urine	acute phase glycoprotein	R-SLEDAI; BILAG2004; SLICC; BAI; clinical parameters	LN activity	Yes	9	7 CS; 2 PC
			LN patients	uNGAL	urine	acute phase glycoprotein	R-SLEDAI; BILAG2004; pBILAG; clinical parameters	LN prediction of flare	Yes	10	3 CS; 7 PC

author (year)	Country	Search date	Population	Index test	Biological sample	Tipo de BM	Reference test	Diagnosis	Meta-analysis	Number of included studies	Study design
			LN patients	uNGAL	urine	acute phase glycoprotein	Renal biopsy	Proliferative LN	Yes	6	PC
Wang, Z. et al. (2020)	China	Until September 2019	SLE patients	TWEAK	urine	cytokine	Renal Biopsy; clinical paranmeters; R-SLEDAI	LN	Yes	11	NI
			SLE patients	TWEAK	urine	cytokine	Renal Biopsy; clinical paranmeters; R-SLEDAI	LN activity	Yes	4	NI
Xia, Y-R. et al. (2020)	China	Until November 2019	LN patients	MCP-1	urine	cytokine	SLEDAI	LN activity	Yes	3	NI
Ma, H. Y. et al. (2021)	China	Until August 2020	SLE patients	TWEAK	urine and serum	cytokine	Renal Biopsy; R-SLEDAI	LN activity	Yes	9	8 CS; 1 PC
Radin, M. et al. (2021)	Italy	April 2015 until April 2020	SLE patients	Novel BM	urine and serum	diverse	SLEDAI; R-SLEDAI; SLEDAI 2000; SLICC/ACR DI; UK; BAI; R-BILAG; SLICC RAS; BILAG; SELENA-SLEDAI; BILAG 2004; SLAM; Renal biopsy; clinical parameters	LN; LN activity; LN prognosis	No	85	62 CS; 14 PC; 4 CC; 4 LS and CS, 1 longitudinal

NI = Not informed; *Clinical parameters = 24h proteinuria, Urine Protein to Creatinine Ratio (UPCR), creatinine, active sediment; [a] R-SLEDAI = Renal-Systemic lupus erythematosus disease activity index; [b] SLEDAI-2000 = Systemic lupus erythematosus disease activity index 2000 ; [c] SLEDAI = Systemic lupus erythematosus disease activity index ; [d] SLICC = The Systemic Lupus International Collaborating Clinics ; [e] BAI = Biopsy activity index ; [f] BILAG 2004 = British Isles Lupus Assessment Group's disease activity index; [g] pBILAG = Pediatric British Isles Lupus Assessment Group index; [h]SLICC/ACR DI = Systemic Lupus International Collaborating CLinics/American College of Rheumatology Criteria Damage Index; [i]R-BILAG = Renal British Isles Lupus Assessment Group; [j]SLICC RAS = The Systemic Lupus International Collaborating Clinics Renal Activity Score; [l]SLAM = Systemic lupus activity measure; [m] CS = Cross-sectional; [n]PC = prospective cohort; [o]BM = biomarkers; [p]CC = case-control; [q]LS = longitudinal study.

Table 2. Summary of principal data of included reviews.

author (year)	Index test	Number of included studies (number of participants)	Subjects age (years)	Diagnosis	Pooled sensitivity	Pooled specificity	PLR	NLR	SROC-AUC	DOR	Heterogeneity	Publication bias
Benito-Garcia, E et al. (2004)	anti-Sm	8 (n=984)	NR	LN	0.25 (0.17 - 0.36)	0.85 (0,78 - 0.91)	1.3	NR	Fez a SROC e não deu o valor da AUC	NR	NR	NR
	anti-RNP	8 (n=1114)	NR	LN	0.28 (0.18 - 0.41)	0.74 (0.65 - 0.81)	1.1	NR	Fez a SROC e não deu o valor da AUC	NR	NR	NR

author (year)	Index test	Number of included studies (number of participants)	Subjects age (years)	Diagnosis	Pooled sensitivity	Pooled specificity	PLR	NLR	SROC-AUC	DOR	Heterogeneity	Publication bias
Yin, Y. et al. (2012)	anti-C1q	22 (n=2381)	9,8 - 43	LN	0,58 (0,56-0,61)	0,75 (0,72 - 0,77)	2,6 (2,06 - 3,28)	0,51 (0,41 - 0,63)	0,7941	6,08 (3,91 - 9,47)	High	Yes
	anti-C1q	9 (n=517)	9,8 - 43	activity	0,74 (0,68 - 0,79)	0,77 (0,71 - 0,82)	2,91 (1,83 - 4,65)	0,33 (0,19 - 0,56)	0,8378	10,56 (4,56 - 24,46)	High	Yes
Eggleton P. et al. (2014)	anti-C1q	31 (28 meta-analysed) (n=2769)	>= 15 anos (15 - 77); pediátrico (mean age 13,9)	LN	0.73	0.70 (0.57 - 0.81)*	2.66	0.40	Fez a SROC e não deu o valor da AUC	NR	NR	NR
	anti-C1q	31 (9 meta-analysed) (n=517)	>= 15 anos (15 - 77); pediátrico (mean age 13,9)	activity	0.80	0.75 (0.46 - 0.91)*	3.79	0.30	Fez a SROC e não deu o valor da AUC	NR	NR	NR
Fang Y. G. et al. (2020)	uNGAL	4 (n=177)	10 - 35	LN	0.73 (0.61 - 0.83)	0.78 (0.69 - 0.85)	3.88 (1.14 - 13.24)	0.36 (0.160 - 0.82)	0.86	14.83	Moderate to High	No
	uNGAL	8 (n=815)	11,6 - 44,1	activity	0.66 (0.60 - 0.71)	0.62 (0.57 - 0.66)	2.05 (1.25 - 3.37)	0.43 (0.22 - 0.86)	0.75	5.46	HIgh	No
	uNGAL	6 (n=442)	14,1 - 41	Prediction of flares	0.77 (0.68 - 0.85)	0.65 (0.60 - 0.70)	2.24 (1.47 - 3.42)	0.37 (0.17 - 0.81)	0.77	6.28	Moderate	No
Wang, Z. et al. (2015)	anti-C1q	11 (n=1084)	9 - 37,1 (+/- 11,9)	LN	0,67 (0,63 - 0,71)	0,69 (0,65 - 0,74)	2,18 (1,75 - 2,72)	0,48 (0,39 - 0,60)	0,749	5,09 (3,29 - 7,85)	Moderate	Yes
Puapatanakul, P; et al. (2019)	Serum IP-10	2 (n=?)	NR	LN	QS	QS	QS	QS	QS	QS	QS	QS
	Urinary IP-10	5 (n=?)	NR	LN	QS	QS	QS	QS	QS	QS	QS	QS
Gao, Y; et al. (2020)	uNGAL	9 (n=573)	11,6 - 35	LN	0,84 (95% CI 0,71 - 0,91)	0,91 (95% CI 0,70 - 0,98)	9,08 (95% CI 2,31 - 35,69)	0,18 (95% CI 0,09 - 0,35)	0,92 (95% CI 0,90 - 0,94)	50,51 (95% CI 8,15 - 313,03)	High	No
	uNGAL	10 (n=949)	11,6 - 44,1	activity	0,72 (0,56 - 0,84)	0,71 (0,51 - 0,84)	2,45 (1,32 - 4,54)	0,39 (0,22 - 0,70)	0,77 (0,74 - 0,81)	6,24 (2,08 - 18,68)	High	No
	uNGAL	6 (n=442)	11,3 - 41	prediction of flare	0,80 (0,57 - 0,92)	0,67 (0,58 - 0,75)	2,41 (1,57 - 3,72)	0,30 (0,11 - 0,79)	0,74 (0,70 - 0,78)	8,08 (2,02 - 32,35)	Moderate	No
	uNGAL	2 (n=36)	10 - 30	Proliferativ e LN	0,87 (0,66 - 0,97)	0,69 (0,39 - 0,91)	2,89 (1,26 - 6,61)	0,20 (0,06 - 0,65)	not constructed	16,42 (2,56 - 105,37)	-	-

author (year)	Index test	Number of included studies (number of participants)	Subjects age (years)	Diagnosis	Pooled sensitivity	Pooled specificity	PLR	NLR	SROC-AUC	DOR	Heterogeneity	Publication bias
Wang, Z. et al. (2020)	TWEAK	4 (n=276)	28 (+/- 11,8) - 35,5 (+/- 12,7)	LN	0,55 (0,47 - 0,63)	0,92 (0,86 - 0,96)	NR	NR	0,8224	16,54 (7,57 - 36,15)	Low	No
	TWEAK	3 (n=139)	25,6 (+/- 10,7) - 32,9 (+/- 10,37)	activity	0,91 (0,82 - 0,96)	0,70 (0,58 - 0,81)	NR	NR	0,8131	18,54 (7,45 - 45,87)	Low	No
Xia, Y-R. et al. (2020)	MCP-1	7 (n=521)	23,66 (+/-4,55) - 36,9 (+/-10,62)	activity	0,89 (0,86 - 0,93)	0,63 (0,55 - 0,69)	2,16 (1,66 - 2,80)	0,15 (0,08 - 0,30)	0,90	19,40 (7,24 - 51,96)	Moderate to High	No
Ma, H. Y. et al. (2021)	TWEAK	9 (n=334)	NR	activity	0,69 (0,63 - 0,75)	0,77 (0,71 - 0,82)	3,31 (2,05 - 5,35)	0,38 (0,26 - 0,55)	0,827	10,89 (6,73 - 17,63)	High	No
Radin, M. et al. (2021)	Novel BM	85 (n=13.496)	NR	LN; activity; prognosis	QS	QS	QS	QS	QS	QS	NR	NR

* median; QS = Qualitative synthesis; NR = Not reported; BM = biomarkers

Biomarkers studied

Autoantibodies

Several autoantibodies have been investigated as possible BMs in LN. We found 4 SRs that evaluated the diagnostic accuracy of the following autoantibodies in LN: anti-Sm, anti-RNP (41) and anti-C1q (42-44).

Anti-Sm and anti-RNP

Anti-Sm and anti-RNP are autoantibodies that target small nuclear ribonucleoproteins (snRNPs); they are among the most commonly used BMs in patients with diagnosed or suspected systemic autoimmune diseases (48).

Anti-Sm is associated with the diagnosis of SLE and is part of the disease classification criteria (49). However, the role of anti-Sm has been investigated in other contexts and has been associated with other clinical variables of the disease, such as pericarditis, CNS involvement and renal involvement (50-53).

Anti-RNP antibodies can be detected in several systemic autoimmune diseases, including SLE. However, its clinical value is found in the strong association of high titres with mixed connective tissue disease (MCTD) (48).

Benito-Garcia, et al. conducted a systematic review to determine the sensitivity, specificity and predictive values of anti-Sm and anti-RNP autoantibodies in the diagnosis of SLE and other related systemic autoimmune diseases and to identify their clinical associations.

Thirteen studies were included in this SR, which evaluated the accuracy of anti-Sm antibodies in the detection of LN. Additionally, 8 of these studies were included in a meta-analysis. The weighted mean sensitivity was 0.25 (95% CI 0.17-0.36), the

specificity was 0.85 (95% CI 0.78-0.91), and the median PLR was 1.3. The corresponding summary receiver operating characteristics (SROC) showed that most of the points were dispersed around the diagonal line, which, together with the reported data, demonstrate the low relevance of this BM as a potential influencer of clinical decision-making.

The 5 studies that were not included in the meta-analysis were qualitatively synthesized (54-58). In 3 of these studies (54, 56, 57), no significant correlation was found between anti-Sm and renal involvement of the disease. One of the studies correlated anti-Sm with WHO Class V nephritis (membranous glomerulonephritis) (55). The other study by Win et al., only 1 of the 23 lupus patients who were positive for anti-Sm presented Class IV nephritis (diffuse proliferative glomerulonephritis), and among the other patients, most presented mesangial, membranous or focal histopathological changes, and 4 had a normal renal biopsy (58).

Eight of the included studies analysed the value of anti-RNP antibodies in the diagnosis of LN. The following weighted mean results were found: Sensitivity was 0.28 (95% CI 0.18-0.41), specificity was 0.74 (95% CI 0.65-0.81), and PLR was 1.1. The SROC also showed the dispersion of the points around the diagonal line, reinforcing the conclusion that this antibody is of little use in the detection of LN.

The quality of the studies was evaluated using the criteria developed by the Evidence-based Medicine Working Group (59), and only studies classified as Grade A and Grade B (high methodological quality) were included in the reviews. However, the presence of heterogeneity among the studies in either of the 2 arms of the SRs was not evaluated, and it was not possible to analyse the impact of such heterogeneity on the results.

Anti-C1q

Although it was initially described in the serum of SLE patients, anti-C1q autoantibodies have been detected in up to 8% of apparently healthy individuals (60) and have been studied in several other autoimmune diseases, infectious diseases and various kidney diseases (61, 62).

In SLE, several studies have associated anti-C1q with renal impairment caused by the disease (25, 63), a finding that has been reinforced by experimental studies demonstrating a possible pathogenic role of this autoantibody in SLE (64, 65).

Three SRs were included that evaluated the role of anti-C1q as a BM in LN (42-44). Two of the SRs analysed the accuracy of anti-C1q for diagnosing LN among SLE patients and for detecting its activity (42, 44). Yin et al. and Eggleton et al. showed partial overlap of the included studies. The review by Eggleton et al. encompassed all the articles that were included in the SR performed by Yin et al. and added 6 additional studies evaluating the accuracy of anti-C1q in the discrimination of patients with a current or previous history of LN (66-71). The 2 reviews showed results in the same direction, although Eggleton found overall accuracy measures higher than those found by Yin (**Table 2**), possibly because Eggleton included additional studies and used different statistical methods to summarize the results. The heterogeneity among the included studies was high in terms of the evaluation of this antibody's accuracy for both the diagnosis of LN and the detection of its activity. No threshold effect was found in any of the analyses, and the covariates that were explored by meta-regression (quality of the study, detection method and ethnic group) did not influence the results. The Egger test, which was applied in the review by Yin, showed a significant probability of publication bias. Despite these limitations, anti-C1q was identified as a potential BM in LN.

The review by Wang et al. included only studies that were conducted in the Chinese population and evaluated the accuracy of anti-C1q in the diagnosis of LN in

patients with SLE. A total of 11 studies were included; of the 1084 patients with SLE included in these studies, 474 were diagnosed with LN. The pooled sensitivity was 0.67 (95% CI 0.63-0.71), the pooled specificity was 0.69 (95% CI 0.65-0.74), the positive likelihood ratio (PLR) was 2.18 (95% CI 1.75-2.72), the negative likelihood ratio (NLR) was 0.48 (95% CI 0.39-0.60), the diagnostic odds ratio (DOR) was 5.09 (3.29-7.85) and the SROC-AUC was 0.749. The heterogeneity among the studies was significant, with I^2 values ranging from 43.6% for PLR to 88.9% for sensitivity. In the subgroup analysis of the possible sources of inconsistency, the methodological quality, the age of the evaluated population and the sample size were considered. However, none of these variables seemed to have a significant influence on heterogeneity, and no threshold effect was observed. Although the review included only studies of Chinese populations, the accuracy values, although lower, were not far from those found in the other 2 reviews, especially for PLR, NLR and DOR (**Table 2**).

The role of anti-C1q as a BM in LN is not yet defined. In the SRs that were identified, it did not perform well for differentiating patients according to a positive or negative test. However, there seems to be a benefit to its use, which may have been obscured by the potential effect of the heterogeneity among the studies.

Cytokines

Tumour necrosis factor-like weak inducer of apoptosis (TWEAK)

TWEAK is a proinflammatory cytokine in the TNF superfamily that activates fibroblast growth factor-inducible 14 (Fn14), a protein in the TNF receptor superfamily that is constitutively present in healthy tissues, and may increase its expression in inflammatory situations (72). TWEAK is secreted mainly by monocytes and macrophages and participates in tissue repair and remodelling (72). Several studies have indicated the involvement of the TWEAK-Fn14 axis in the pathogenesis of chronic

autoimmune diseases, especially in cases of neurological, vascular and renal involvement (73).

Two systematic reviews focused on the diagnostic performance of TWEAK as a BM for lupus nephritis (46, 74). The SR by Wang et al. (46) addressed the role of urinary TWEAK (uTWEAK) as a BM in LN, evaluating its accuracy in the diagnosis of LN in patients with SLE and in the detection of LN activity. The analysis of the diagnostic accuracy of TWEAK for the detection of LN involved 7 studies and resulted in a pooled sensitivity of 0.55 (95% CI 0.47-0.63), a pooled specificity of 0.92 (95% CI 0.86-0, 96), a DOR of 16.54 (95% CI 7.57-36.15) and an SROC-AUC of 0.822.

Regarding its diagnostic accuracy in the detection of nephritis, the pooled sensitivity was 0.91 (95% CI 0.82-0.96), the pooled specificity was 0.70 (95% CI 0.58-0.81), the DOR was 18.54 (7.45-45.87) and the SROC-AUC was 0.813. Despite the small number of primary studies included in both arms of the review, the heterogeneity among them was low, and no threshold effect was observed.

Ma et al. (74) reviewed primary studies assessing the diagnostic accuracy of serum and urinary TWEAK in predicting active LN in SLE patients. Nine studies were included, 7 of which evaluated TWEAK in urine and 2 in serum (sTWEAK).

The summarized data revealed a pooled sensitivity of 0,69 (95%CI 0,63 – 0,75), pooled specificity of 0,77 (95%CI 0,71 – 0,82), pooled positive likelihood ratio of 3,31 (95%CI 2,05 – 5,35), pooled negative likelihood ratio of 0,38 (95% CI 0,26 – 0,55), pooled DOR of 10,89 (95%CI 6,73 – 17,63) and a ROC/AUC of 0,827 (SE 0,0289). The heterogeneity among the studies was moderate to high. The subgroup analysis revealed that the pooled sensitivity, DOR and AUC of TWEAK in predicting active LN were higher in patients with R-SLEDAI > 4 when compared to patients with R-SLEDAI > 0 (0,85 x 0,66; 19,00 x 8,90 and 0,90 x 0,79 respectively). uTWEAK also revealed a higher pooled DOR than sTWEAK (12,4 and 6,76, respectively). Moreover, TWEAK and R-SLEDAI

were correlated in 6 of the studies; and in 5 of them, the correlation was between TWEAK and proteinuria.

Despite the review of Ma et al. being more recent and with a more significant number of studies, the overlapping of primary studies among both included SRs on TWEAK comprised a total of 5 works (75-79), amounting to 7 the number of non-overlapping studies (79-85) (6 evaluating uTWEAK and 2 sTWEAK).

Regardless of the methodological differences between both SRs, the partial intersection of primary studies, and their heterogeneity, the results point to uTWEAK as an auspicious BM for the clinical management of LN.

Chemokines

Monocyte chemoattractant protein-1 (MCP-1)

MCP-1 is a chemokine in the CC family that is composed of 76 amino acids and is produced by epithelial cells, endothelial cells, smooth muscle cells, monocytes, macrophages, fibroblasts, astrocytes and microglial cells under various stimuli, such as oxidative stress, cytokines and growth factors (86). MCP-1 has been implicated in the pathogenesis of several diseases through its influence on chemotaxis and oxidative stress, among other actions (87-89). In SLE, MCP-1 has been associated with disease activity and renal impairment (90, 91).

Only 1 SR was found on the use of MCP-1 as a BM in LN (47). Xia et al. analysed primary studies, evaluating their diagnostic accuracy in detecting renal disease activity. Seven studies with a total of 521 participants were included. The pooled sensitivity was 0.89 (95% CI 0.86-0.93), the pooled specificity was 0.63 (95% CI 0.55-0.69), the PLR

was 2.16 (95% CI 1.66-2.80), the NLR was 0.15 (95% CI 0.08-0.30), the DOR was 19.4 (95% CI 7.24-51.96) and the SROC-AUC was 0.90.

There was high heterogeneity among the studies, with an I^2 of 75.4%. There was no threshold effect, and in a subgroup analysis, ethnicity and the presence of inactive LN had no influence on the inconsistency that was observed; however, no sensitivity analysis was performed. There was no evidence of publication bias. Despite the limitations of the data, MCP-1 seems to be superior to the conventional serological BMs used in the management of LN.

Interferon inducible protein-10 (IP-10)

IP-10 or CXCL10 is a chemokine in the ELR$^-$CXC family that is produced by T lymphocytes, natural killer (NK) cells, NK-T cells, neutrophils, monocytes, splenocytes, endothelial cells, fibroblasts, keratinocytes and other types of cells under the stimulus of proinflammatory cytokines (92). It has chemotactic power over lymphocytes, participates in the regulation of cell growth and has angiostatic properties (93, 94). The role of IP-10 has been studied in several autoimmune diseases, such as rheumatoid arthritis (95), Sjögren's syndrome (96) and multiple sclerosis (97). In SLE patients, studies have shown high levels of IP-10 in serum (98) and in samples from cutaneous lesions of the disease (99), and it appears to correlate with disease activity (100).

Puapatanakul et al. conducted a systematic review of studies that evaluated the serum and urinary levels of IP-10 in patients with SLE with and without LN. A total of 23 publications were included, and only 6 evaluated IP-10 specifically in LN. Most of the included studies did not evaluate diagnostic accuracy measures. The meta-analysis consisted of values that referred to mean differences between the studied groups; these showed no statistical significance of the serum IP-10 for differentiating between patients

with LN and patients with SLE without nephritis, only a tendency toward higher urinary concentrations in patients with LN than in patients without LN.

Only 2 studies evaluated the diagnostic accuracy of serum IP-10 levels for the detection of renal involvement in patients with SLE; however, no meta-analysis was performed. The studies presented an analysis of the area under the ROC curve (ROC-AUC), showing values ranging from 0.596 to 0.633, emphasizing the lack of utility for this outcome.

Among the studies that evaluated the urinary levels of IP-10 for the detection of renal involvement, only 5 studies analysed the ROC curve to demonstrate its overall performance. One of the studies showed an area under the ROC curve (ROC-AUC) of 1.000 (101). In 3 other studies (80, 102, 103), the urinary IP-10 showed ROC-AUCs ranging from 0.595 to 0.680, which was not superior the findings for conventional BMs.

In 1 of the included studies, urinary IP-10 levels were measured by mRNA detection by RT–PCR, and urinary IP-10 showed a good ability to distinguish Class IV from LN (diffuse proliferative glomerulonephritis), with a sensitivity of 0.73, a specificity of 0.94 and an ROC-AUC of 0.89 (95% CI 0.78-0.99) (104). However, the number of patients evaluated was small (26 subjects).

It was not possible to reach a conclusion regarding the diagnostic accuracy of IP-10 in LN. There was considerable disagreement among the diagnostic accuracy measures used the various primary studies, the number of studies that evaluated this aspect was small, and the population samples were also small. The SR of Puapatanakul found no difference between the mean serum levels of IP-10 in patients with active LN, those of patients with active SLE without LN and those of patients with inactive LN. Regarding urine levels, only a statistically significant tendency was found for these to be higher in patients with nephritis; however, the heterogeneity among the studies was high.

Other molecules

Neutrophil gelatinase-associated lipocalin (NGAL)

NGAL is an acute phase glycoprotein belonging to the lipocalin family. Under conditions of homeostasis, it is secreted by neutrophils, macrophages, hepatocytes, adipocytes, neurons and epithelial cells, and its production is significantly increased under inflammatory stimulus, oxidative stress and tissue injury (105, 106). Several studies have associated increased urinary NGAL concentrations with various types of kidney injury (107-109). In SLE, an *in vitro* study by Qing et al. showed increased expression of Lipocalin-2 in mesangial cells derived from SLE patients after stimulation with anti-murine DNA antibody (110), and observational studies conducted in humans have shown higher urinary concentrations of NGAL in patients with LN (111, 112).

Two SRs evaluated the role of NGAL as a BM in LN (37, 45). The review by Gao et al. is more recent (2020) and encompasses all of the primary studies evaluated by Fang et al. plus 8 additional articles, for a total of 19 articles. The evaluated outcomes were the accuracy of uNGAL in the diagnosis of LN, the detection of LN activity, the prediction of LN relapse and the distinction between the proliferative and non-proliferative forms of LN (the latter outcome was evaluated only in the Gao review). The 2 SRs identified results in the same direction for the accuracy of NGAL, although the most relevant results were its accuracy for the diagnosis of LN in SLE patients and the summary measures reported by Gao, which are encouraging (**Table 2**) and are described next.

The 19 articles included in Gao et al. corresponded to 21 studies and a total of 1453 participants, including both adults (17 studies) and children (4 studies). The main method for the detection of uNGAL was ELISA, which was used in all primary studies except for 1 (113), which used a chemiluminescent microparticle (CMIA) immunoassay.

The reference tests varied between the various studies and depending on the outcomes studied, as shown in **Table 2**.

Regarding the diagnosis of LN, data from 9 studies (76, 111, 112, 114-119) were evaluated. The pooled sensitivity was 0.84 (95% CI 0.71-0.91), the pooled specificity was 0.91 (95% CI 0.70-0.98), the pooled PLR was 9.08 (95% CI 2.31-35.69), the pooled NLR was 0.18 (95% CI 0.09-0.35), the DOR was 50.51 (95% CI 8.15-313.03) and the area under the SROC curve (SROC-AUC) was 0.92 (95% CI 0.90-0.94).

Ten studies (111, 114, 120-126) analysed the diagnostic accuracy for detecting kidney disease activity. The pooled sensitivity was 0.72 (95% CI 0.56-0.84), the pooled specificity was 0.71 (95% CI 0.51-0.84), the pooled PLR was 2.45 (95% CI 1.32-4.54), the pooled NLR was 0.39 (0.22-0.70), the DOR was 6.24 (95% CI 2.08-18.68) and the SROC-AUC was 0.77 (95% CI 0.74-0.81).

The diagnostic accuracy for predicting LN relapse was evaluated in 6 studies (113, 121, 124, 126). The pooled sensitivity was 0.80 (95% CI 0.57-0.92), the pooled specificity was 0.67 (95% CI 0.58-0.75), the pooled PLR was 2.41 (95% CI 1.57-3.72), the pooled NLR was 0.30 (95% CI 0.11-0.79), the DOR was 8.08 (95% CI 2.02-32.35) and the SROC-AUC was 0.74 (95% CI 0.70-0.78).

There was high heterogeneity among the studies for all outcomes evaluated, with I^2 values ranging from 66.15% to 94.24%. In the meta-regression, subgroup and sensitivity analyses, a possible influence of the quality of the study (defined by the QUADAS-2 score) on accuracy RO4 the diagnosis of LN among patients with SLE was identified. The higher-quality studies (QUADAS-2 ≥13) showed lower pooled sensitivity and higher pooled specificity than the lower-quality studies. The design of the study also showed an influence on the results of the synthesis of accuracy for the detection of LN activity, with the cross-sectional studies showing higher pooled sensitivity and specificity

values than the prospective cohort studies. The reference test that was used had an influence on accuracy for the prediction of relapses, with the studies that used R-SLEDAI showing higher pooled sensitivity and specificity and lower heterogeneity (a pooled sensitivity of 0.80 to 0.90, a pooled specificity of 0.67 to 0.74 and I^2 values of 72.5% to 55.4% and 66.15% to 21.17%, respectively). However, the influence of the examined variables was partial, and other sources of influence were not identified. There was no threshold effect in any of the evaluated outcomes, and there was no evidence of publication bias.

One of the included SRs evaluated the clinical usefulness of novel serological and urinary biomarkers in LN during the last five years (127). Radin et al. SR resulted in the inclusion of 85 studies with varied designs and sample sizes. 13.496 patients with SLE were included, and 79 distinct urinary biomarkers and 80 serum biomarkers were evaluated. A particular emphasis was given to the most studied biomarkers: Monocyte Chemoattractant Protein-1 (MCP-1) (18 studies), Urinary Vascular Cell Adhesion Protein 1 (VCAM-1) (5 studies), Urinary Adiponectin (AdipoQ) (5 studies), Urinary Kidney Injury Molecule-1 (Kim-1) (5 studies), Urinary TNF-like Weak Inducer of Apoptosis (TWEAK) (5 studies) and Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) (8 studies).

The study designs of the included studies were cross-sectional (62 studies), followed by prospective cohort (14 studies), case-control (4 studies), longitudinal and cross-sectional (4 studies), and longitudinal (1 study). Forty-four studies (52%) analyzed only serological BMs, 29 studies (34%) only urinary BMs, 5 studies (6%) examined BMs in both biological materials, and 5 studies also evaluated the studied biomarker in the renal biopsy specimens.

Despite the critical information provided by this SR, there was no qualitative synthesis of the evidence, no report of the evaluated endpoints (e.g. MD, sensitivity, specificity, DOR), or the risk of bias in the included studies.

Methodological quality of the included reviews

The results of the evaluation with the ROBIS tool showed that 6 of the 9 reviews had a low overall risk of bias. The included SRs presented their research questions in a way that was compatible with this overview. However, some were more comprehensive and did not have clearly defined PIRD components. The domains that most frequently presented risk of bias were those related to eligibility criteria and to the identification and selection of studies. None of the reviews reported the registration of a previous protocol, 4 presented restrictions of the inclusion of studies without justification (e.g., quality, language, etc.), 6 did not clearly report whether free or controlled terms were included in the search strategy, 6 did not include grey literature, and 2 did not use at least two reviewers throughout the review process. All of the SRs used some tool to analyse the quality of the included primary studies or their risk of bias, and QUADAS and QUADAS-2 were the most frequently used tools. Most of the SRs considered the methodological quality and/or risk of bias of the included primary studies when interpreting the summarized results. The risk of bias of the included SRs, evaluated by the ROBIS tool, is shown graphically in **Figures 2 and 3**.

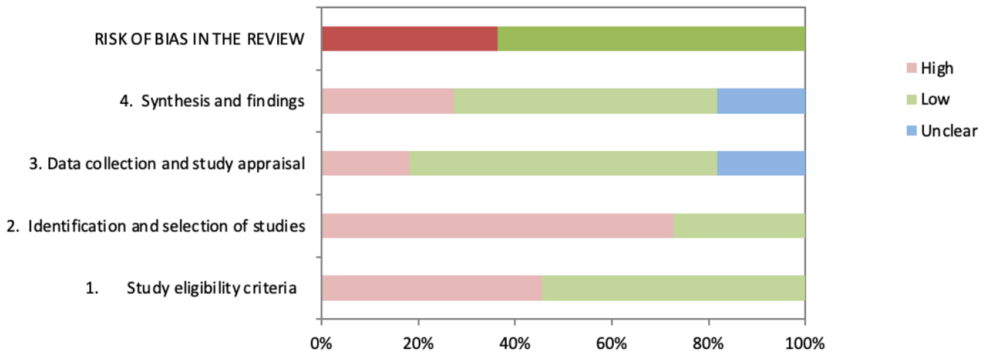


Fig 2. Risk of bias assessment with ROBIS tool.

Darker colours indicate overall ROBIS rating; lighter colours concern judgments.

Table 3. Risk of bias assessment with ROBIS tool.

Review	Phase 2				Phase 3
	1. Study eligibility criteria	2. Identification and selection of studies	3. Data collection and study appraisal	4. Synthesis and findings	Risk of bias in the review
Benito-Garcia 2004	☺	☹	☹	☹	☺
Yin 2012	☹	☹	☺	☺	☺
Eggleton 2014	☺	☺	☺	☺	☺
Fang 2015	☺	☺	?	☺	☺
Wang 2015	☺	☹	☺	☺	☹
Puapatanakul 2019	☹	☹	?	☹	☹
Gao 2020	☺	☺	☺	☺	☺
Wang 2020	☹	☹	☺	☺	☺
Xia 2020	☹	☹	☺	?	☹
Ma 2021	☺	☹	☺	?	☺
Radin 2021	☹	☹	☹	☹	☹

☺ = low risk; ☹ = high risk; ? = unclear risk

Discussion

LN is one of the most relevant impairments in SLE because it has a significant prevalence among patients (30 to 60%) and a great impact on prognosis. Regardless of advances in treatment, approximately 10% of patients still progress to end-stage renal disease in the first 5 years after diagnosis and have a risk of death 8 times higher than that of the general population (128).

Although the term “lupus nephritis” gives the impression of a single type of lesion, it comprises a diverse set of kidney injuries that can compromise any of the tissue compartments of the kidney with varying degrees of association; this results in clinical manifestations of variable severity and evolution (129), which makes the discovery of good BMs a great challenge.

This overview found 9 SRs that addressed the diagnostic accuracy of new serum and urinary BMs in LN. Among these, the following BMs were evaluated: (a) antibodies (anti-Sm, anti-RNP and anti-C1q) (41-44), (b) cytokines (TWEAK and MCP-1) (46, 47), (c) a chemokine (IP-10) (39) and (d) an acute phase glycoprotein (NGAL) (37, 45), as previously described.

The SRs identified mainly evaluated primary studies that answered questions about the accuracy of the BM for the diagnosis of LN in patients with SLE and for the

detection of LN activity. Only the 2 SRs on uNGAL (37, 45) also evaluated studies of the accuracy of the BMs for predicting LN relapse, and only the review of Gao et al. analysed studies of the accuracy of a BM (NGAL) for distinguishing the histopathological type (proliferative and non-proliferative LN) (37).

Anti-Sm and anti-RNP showed to be of no use in the detection of LN (41). Although the SR by Benito-Garcia et al. included primary studies that were of good methodological quality and that evaluated a significant number of individuals (984 for anti-Sm and 1114 for anti-RNP), its search was restricted to studies reported in English and included only 2 databases, which overlap (PubMed and Medline). This confers a reasonable risk that relevant studies were not included. In addition, the confidence intervals for the summarized sensitivity and specificity values were wide. Thus, despite the possibility that these antibodies are not useful as BMs in LN, a more sensitive search would provide a definitive answer regarding their role in this type of SLE impairment.

Only one SR that studied IP-10 was found (39), and it evaluated studies that considered serum and urinary levels of this BM. Five studies were included in the review arm that evaluated serum IP-10. However, only 2 studies performed ROC curve analyses (without meta-analysis), and those showed a poor performance of the BM for detecting nephritis among patients with SLE. Additionally, the meta-analysis of the mean differences (MD) between patients with active LN and patients with SLE without nephritis in the 5 included studies showed no difference. This difference was only significant when patients were compared with healthy controls (as in 3 of the studies).

On the other hand, of the 6 included studies that evaluated urinary IP-10, 5 reported accuracy data with ROC curve analyses. The results were varied but pointed in the same direction, indicating a probable benefit of urinary IP-10 as a BM. However, the review did not provide a quantitative synthesis of these results. It only reported the mean differences (of 3 of the included primary studies), which showed a tendency for the mean

urinary levels of IP-10 to be higher among patients with nephritis. Thus, although the results for serum IP-10 are not encouraging, urinary IP-10 seems to have relevance as a BM in LN and is deserving of further studies.

The BMs with the best accuracy profile were uMCP-1, uTWEAK, uNGAL and anti-C1q, which were more sensitive than specific for the analysed outcomes (37, 42-47). The best sensitivity values were found for the accuracy of detection of nephritis activity. This finding may have been favoured by the fact that these studies compared clearly inflamed subjects (those with active LN) with groups of individuals with clinically inactive disease (with no or little inflammation). This made the composition of each group more homogeneous and, clinically, more distinct from each other, which tended to increase the differences between them.

The sensitivity of a BM varies not only according to test cut-off used but according to the severity of the disease (130). In the context of LN, other factors, such as the affected renal compartments (mesangial, interstitial, vascular, glomerular or tubules), the predominant location of the immune complex deposit (subendothelial or subepithelial), the type of pathological lesion (proliferative or not proliferative) and the established degree of chronicity, are also likely to influence the performance of accuracy measures of the BM being tested.

Thus, an important consideration in the study of BMs in the context of LN is the stratification of patients by (a) the presence of disease activity, (b) clinical severity, (c) histopathological features, (d) the mean time of kidney disease and (e) treatment. This would require a large population sample, which may be more feasible for multicentre research collaborations, and the standardization of smaller studies in terms of the details of the research design used to study diagnostic accuracy in LN. Such efforts could facilitate the subsequent summarization of results and accelerate progress in this area of knowledge.

In this overview, the SRs that were included did not explore the composition of each comparison group within the primary studies in depth. The proportion of individuals with active disease and the histopathological class of nephritis were not discussed in most of the reviews, and these variables may have significantly influenced the heterogeneity of the summarized results.

Another relevant issue was the design of the primary studies. Many diagnostic accuracy studies have a cross-sectional design, which may overestimate the findings when there are individuals in the sample with the disease in different clinical stages or when the reference test is not 100% accurate (131). In the SR of Gao (37), the sensitivity of uNGAL was lower in the arm of the review that evaluated its accuracy for the detection of activity. During the analysis of heterogeneity, it was observed that the cohort studies decreased the pooled sensitivity (compared to the cross-sectional studies).

Cohort studies would most likely generate accuracy measures closer to reality in this context. Renal biopsy (the gold standard for diagnosis) is not repeated regularly as a matter of clinical routine because of its invasive and risky nature. Instead, the detection of renal impairment relies on laboratory tests and activity scoring tools (e.g., R-SLEDAI). This restricts the evaluation of new BMs because their accuracy may be underestimated due to the limitations of the reference tests.

Thus, cohort studies with pre-programmed biological material collection would allow a correct evaluation of the accuracy of the index test, as it would be assessed at various times until evident kidney disease occurs. Among the SRs included in this overview, only 2 reported the design of the included primary studies, which made it difficult to interpret the summarized data.

Another relevant point is the use of BM panels. The histopathological and pathophysiological diversity of LN requires a set of BMs that reflect the various phenomena in progress in renal tissue. Despite the significant heterogeneity of the

results summarized in the included SRs and the limitations that are already known as a result of accuracy studies, the data found in this overview highlight urinary MCP-1, TWEAK, NGAL and anti-C1q as useful BMs in LN, and the inclusion of these in a diagnostic panel offers a promising research approach with existing initiatives (132-136).

This is the first overview to synthesize the existing evidence reported by SRs of the diagnostic accuracy of new serum and urinary BMs in LN. With more than 30 BMs undergoing research in this field and the ongoing discovery of new potential BMs, the synthesis of the existing evidence provides an objective view of the direction of the data on studied BMs and unveils the best paths to followed in related research.

Our overview had a wide scope, including 6 databases, grey literature and no time or language restrictions. However, despite the advantage of providing a panoramic and objective view of the existing evidence on a subject, the results of an overview are subject to failures arising from the handling of secondary data. In our overview, some SRs restricted their search to the English language, used few databases and did not include grey literature, which may have led to the loss of relevant studies.

In addition, none of the SRs had previously registered their protocols, and some did not report the involvement of at least 2 reviewers in all phases of the review, which increases the chance of errors and ad hoc changes that can lead to spurious results. In addition, the heterogeneity among the primary studies, a common problem of SRs and overviews, as well as the variability in the statistical methods used to summarize the data among the SRs, requires careful interpretation.

Conclusion

Our results are relevant because they highlight that among the many BMs that have been studied in LN, urinary MCP-1, TWEAK and NGAL and anti-C1q deserve additional research attention, preferably with standardized methods and LN diagnostic

panels, to obtain a better understanding of their usefulness and possibly validate their clinical use in the future.

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ANEXO 1 – Checklist PRISMA 2020

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	line 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	line 22
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	line 72
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	line 133
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	line 142
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	line 180
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	line 185
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	line 188
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	line 195
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	line 195
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	line 218
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	line 203
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	line 203
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	line 203
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	line 212
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	

PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	line 221
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	line 226
Study characteristics	17	Cite each included study and present its characteristics.	line 229
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	line 538
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	line 230
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	line 561
	23b	Discuss any limitations of the evidence included in the review.	
	23c	Discuss any limitations of the review processes used.	
	23d	Discuss implications of the results for practice, policy, and future research.	
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	line 138
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

ANEXO 2 - S1 Table. Data bases and search strategies used.

Data base	Search strategy
Pubmed	(("Biomarkers"[Mesh] OR "Biomarker*" OR "Biologic Marker*" OR "Biological Marker*" OR "Laboratory Marker*" OR "Serum Marker*" OR "Surrogate End Point*" OR "Surrogate Endpoint*" OR "Clinical Marker*" OR "Viral Marker*" OR "Biochemical Marker*" OR "Immune Marker*" OR "Immunologic Marker*" OR "Surrogate Marker*" OR "salivary biomarker*" OR "urinary biomarker*" OR "blood biomarker*" OR "Serum biomarker*") OR ("Diagnosis"[Mesh:NoExp] OR "diagnosis" OR "diagnoses" OR "diagnostic" OR "diagnosable" OR "diagnose" OR "diagnosed" OR "diagnosing" OR "screening" OR "monitoring" OR "monitor" OR "test" OR "tests" OR "detection" OR "detecting" OR "signs")) AND ("Lupus Nephritis"[Mesh] OR "Lupus Nephritis" OR "Lupus Glomerulonephritis" OR "Lupus Nephritides")
EMBASE	('biomarker'/exp OR biomarker OR 'biomarkers'/exp OR 'biomarkers' OR 'biologic marker' OR 'biological marker'/exp OR 'biological marker' OR 'laboratory marker' OR 'serum marker' OR 'surrogate end point'/exp OR 'surrogate end point' OR 'surrogate endpoint'/exp OR 'surrogate endpoint' OR 'clinical marker' OR 'viral marker' OR 'biochemical marker'/exp OR 'biochemical marker' OR 'immune marker' OR 'immunologic marker' OR 'surrogate marker'/exp OR 'surrogate marker' OR 'salivary biomarker' OR 'urinary biomarker' OR 'blood biomarker' OR 'serum biomarker' OR 'biologic markers' OR 'biological markers'/exp OR 'biological markers' OR 'laboratory markers' OR 'serum markers' OR 'surrogate end points' OR 'surrogate endpoints' OR 'clinical markers' OR 'viral markers' OR 'biochemical markers' OR 'immune markers' OR 'immunologic markers' OR 'surrogate markers' OR 'exhaled condensate biomarkers' OR 'salivary biomarkers' OR 'urinary biomarkers' OR 'blood biomarkers' OR 'serum biomarkers' OR 'diagnosis'/exp OR diagnosis OR diagnoses OR 'diagnostic'/exp OR diagnostic OR diagnosable OR diagnose OR diagnosed OR diagnosing OR 'screening'/exp OR screening OR 'monitoring'/exp OR monitoring OR 'monitor'/exp OR monitor OR 'test'/exp OR test OR tests OR 'detection'/exp OR detection OR detecting OR signs) AND ('lupus nephritis'/exp OR 'lupus nephritis' OR 'lupus glomerulonephritis'/exp OR 'lupus glomerulonephritis' OR 'lupus nephritides')
WOS	TS=(Biomarker* OR "Biologic Marker*" OR "Biological Marker*" OR "Laboratory Marker*" OR "Serum Marker*" OR "Surrogate End Point*" OR "Surrogate Endpoint*" OR "Clinical Marker*" OR "Viral Marker*" OR "Biochemical Marker*" OR "Immune Marker*" OR "Immunologic Marker*" OR "Surrogate Marker*" OR "salivary biomarker*" OR "urinary biomarker*" OR "blood biomarker*" OR "Serum biomarker*" OR diagnosis OR diagnoses OR diagnostic OR diagnosable OR diagnose OR diagnosed OR diagnosing OR screening OR monitoring OR monitor OR test OR tests OR detection OR detecting OR signs) AND TS=("Lupus Nephritis" OR "Lupus Glomerulonephritis" OR "Lupus Nephritides")

Scopus	TITLE-ABS-KEY("Lupus Nephritis" OR "Lupus Glomerulonephritis" OR "Lupus Nephritides") AND TITLE-ABS-KEY(Biomarker* OR "Biologic Marker*" OR "Biological Marker*" OR "Laboratory Marker*" OR "Serum Marker*" OR "Surrogate End Point*" OR "Surrogate Endpoint*" OR "Clinical Marker*" OR "Viral Marker*" OR "Biochemical Marker*" OR "Immune Marker*" OR "Immunologic Marker*" OR "Surrogate Marker*" OR "salivary biomarker*" OR "urinary biomarker*" OR "blood biomarker*" OR "Serum biomarker*" OR diagnosis OR diagnoses OR diagnostic OR diagnosable OR diagnose OR diagnosed OR diagnosing OR screening OR monitoring OR monitor OR test OR tests OR detection OR detecting OR signs)
Lilacs	tw:((tw:(biomarker* OR "Biologic Marker*" OR "Biological Marker*" OR "Laboratory Marker*" OR "Serum Marker*" OR "Surrogate End Point*" OR "Surrogate Endpoint*" OR "Clinical Marker*" OR "Viral Marker*" OR "Biochemical Marker*" OR "Immune Marker*" OR "Immunologic Marker*" OR "Surrogate Marker*" OR "salivary biomarker*" OR "urinary biomarker*" OR "blood biomarker*" OR "Serum biomarker*" OR diagnosis OR diagnoses OR diagnostic OR diagnosable OR diagnose OR diagnosed OR diagnosing OR screening OR monitoring OR monitor OR test OR tests OR detection OR detecting OR signs OR diagnóstico OR diagnose OR rastreamento OR sinais OR triagem OR teste OR detecção OR monitoramento OR diagnosis OR rastreo OR señales OR tamizaje OR prueba OR detección OR monitorización OR monitoreo OR biomarcadores OR "Marcadores Biológicos" OR "Marcadores Bioquímicos" OR "Marcadores Clínicos" OR "Marcadores Imunológicos" OR "Marcadores Substitutos" OR "Marcadores Séricos" OR "Marcadores Virais" OR "Marcadores de Laboratorio" OR "Marcadores de Soro" OR "Marcadores Inmunológicos" OR "Marcadores Sustitutos" OR "Marcadores Virales")) AND (tw:("Lupus Nephritis" OR "Lupus Glomerulonephritis" OR "Lupus Nephritides" OR "Nefrite Lúpica" OR "Glomerulonefrite Lúpica" OR "Nefritis Lúpica"))) AND (db:("LILACS"))
Cochrane	(Biomarker* OR "Biologic Marker*" OR "Biological Marker*" OR "Laboratory Marker*" OR "Serum Marker*" OR "Surrogate End Point*" OR "Surrogate Endpoint*" OR "Clinical Marker*" OR "Viral Marker*" OR "Biochemical Marker*" OR "Immune Marker*" OR "Immunologic Marker*" OR "Surrogate Marker*" OR "salivary biomarker*" OR "urinary biomarker*" OR "blood biomarker*" OR "Serum biomarker*" OR diagnosis OR diagnoses OR diagnostic OR diagnosable OR diagnose OR diagnosed OR diagnosing OR screening OR monitoring OR monitor OR test OR tests OR detection OR detecting OR signs):ti,ab,kw AND ("Lupus Nephritis" OR "Lupus Glomerulonephritis" OR "Lupus Nephritides"):ti,ab,kw

OpenGrey	("Lupus Nephritis" OR "Lupus Glomerulonephritis" OR "Lupus Nephritides") AND (Biomarker* OR "Biologic Marker*" OR "Biological Marker*" OR "Laboratory Marker*" OR "Serum Marker*" OR "Surrogate End Point*" OR "Surrogate Endpoint*" OR "Clinical Marker*" OR "Viral Marker*" OR "Biochemical Marker*" OR "Immune Marker*" OR "Immunologic Marker*" OR "Surrogate Marker*" OR "salivary biomarker*" OR "urinary biomarker*" OR "blood biomarker*" OR "Serum biomarker*" OR diagnosis OR diagnoses OR diagnostic OR diagnosable OR diagnose OR diagnosed OR diagnosing OR screening OR monitoring OR monitor OR test OR tests OR detection OR detecting OR signs)
ProQuest (thesis and dissertations)	noft(Biomarker* OR "Biologic Marker*" OR "Biological Marker*" OR "Laboratory Marker*" OR "Serum Marker*" OR "Surrogate End Point*" OR "Surrogate Endpoint*" OR "Clinical Marker*" OR "Viral Marker*" OR "Biochemical Marker*" OR "Immune Marker*" OR "Immunologic Marker*" OR "Surrogate Marker*" OR "salivary biomarker*" OR "urinary biomarker*" OR "blood biomarker*" OR "Serum biomarker*" OR diagnosis OR diagnoses OR diagnostic OR diagnosable OR diagnose OR diagnosed OR diagnosing OR screening OR monitoring OR monitor OR test OR tests OR detection OR detecting OR signs) AND noft("Lupus Nephritis" OR "Lupus Glomerulonephritis" OR "Lupus Nephritides")